

nature

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Did Dr Shah die in vain?

THREE years ago Dr V. H. Shah, a senior agronomist with the Indian Agricultural Research Institute (IARI), committed suicide in protest against the system prevailing in Indian agricultural research—and his was the third suicide of an agriculturalist in twelve years. His complaints, left in a letter to the director of IARI Dr Swaminathan, fell into two categories—first, that scientific results were being misrepresented to the extent that misleading and incorrect publicity was being disseminated; and second, that the career structure in agricultural research was being undermined by improper appointments. The failure of Dr Shah to be appointed to a professorship in agronomy was the immediate cause of his suicide, and in his letter he mentioned another appointment in agronomy which he also regarded as going to the wrong man.

Reaction was rapid. A committee of considerable eminence was established and reported in 1973. Since there were obviously more complaints in the air against the Indian Council for Agricultural Research (ICAR) than Dr Shah had spelt out, the committee investigated recruitment files and sent out questionnaires to a wide range of scientists.

To the accusation of scientific misrepresentation, the committee gave a mixed response. Dr Shah had named three specific cases, and in only one of the three did the committee go along with his accusation, although there were some sharp words on the way that advances in agricultural science were being publicised, and this criticism extended beyond the instances raised in the suicide note. The scientific issues have been rather extensively aired in *New Scientist* recently and need no further comment here.

The question of employment conditions and morale are, however, worth discussion. The committee unearthed much dissatisfaction and hard feeling within the agricultural community. This was directed at just about every aspect in their scientific life: appointments were said to be going to incompetents, library facilities were inadequate, there was too much power at headquarters, it was impossible to promote without there being a vacancy available (and competition for it) and equally impossible to get rid of incompetent workers. Perhaps most significantly, half the responses to the questionnaire on the question of what caused most difficulty in doing research blamed interference by supervisors.

The examining committee accepted the validity of much of this criticism. Indeed, the committee's own technical advisers themselves felt sufficiently strongly to write that the malaise which they had observed was not confined to ICAR, but "barring minor exceptions, pervaded the entire scientific and academic community of the

country. At root, it is greed for bureaucratic power and love of a comfortable life which afflicts this class. Juniors are intellectually as corrupt as seniors, and politicisation of academic and scientific life makes matters much worse."

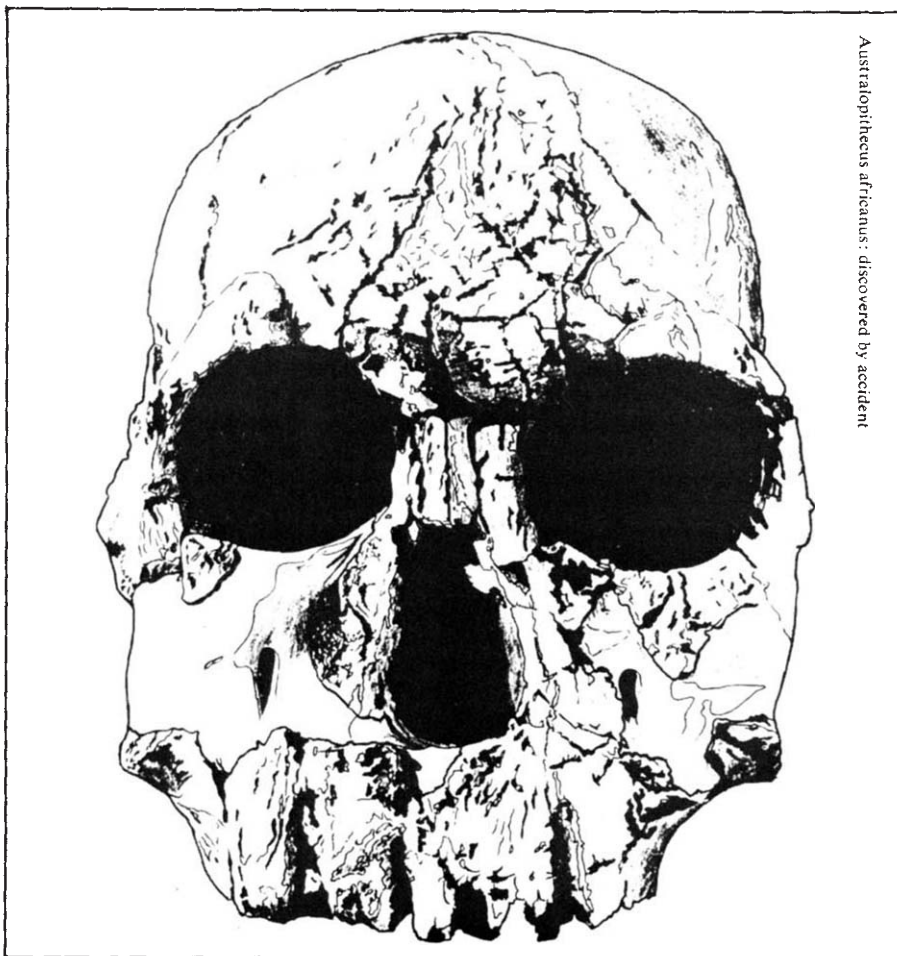
Ironically the committee were not so impressed by two of the cases brought forward by Dr Shah himself. They found that the appointment which he had himself hoped to secure and which went to someone else was unobjectionable. And whilst they were critical of a second appointment made a few months earlier (Dr Shah had not applied for this post) there was considerable disagreement on whether a Ph.D in crop physiology plus ten years in agronomy was a suitable qualification for a post advertised as requiring a Ph.D in agronomy plus ten years' experience.

The almost obsessively narrow nature of this issue unfortunately provided a rather simple escape mechanism—better procedures in recruitment and promotion in ICAR. Unfortunate for two reasons—first because it is by no means clear that Indian science, already much stifled by its reverence for degrees, authority and seniority, needs even more rigid adherence to paper qualifications (a third of all who responded to the committee's questionnaire said that promotion should be based on seniority, without reference to merit). The best science doesn't always reveal itself to the best qualified. Second, because there is still no real sign that the Indian government sees more than administrative changes as necessary as a consequence of Dr Shah's suicide and this report. As the technical advisers bluntly put it, intellectual corruption exists at all levels; much more than changes in selection procedures are needed to eliminate it. The origins of this corruption are not difficult to find—they lie in the surfeit of bureaucracy, concentration of power at headquarters, a gross oversupply of graduates some of whom seem to acquire their degree only for matrimonial purposes, the shortage of money, the poor quality of technical help and the lack of diversity of opportunity within Indian science.

None of these problems is easily solved, but they are all potentially soluble. Mrs Gandhi, by being Minister for Science and Technology herself, is expressing a national faith that science and technology can still help India. She is in a powerful position to take bold steps to reduce mistrust and discontent amongst the scientific community, and she should take radical action.

We regret the poor quality of the paper on which last week's *Nature* was published. Continuity of paper supply has posed a continual problem for us, but we are doing everything possible to stabilise the situation.





Australopithecus africanus: discovered by accident

It is almost 50 years to the day that Nature published a report by Dr Raymond Dart describing the face, jaw and the brain case of a child belonging to what Dart described as "an extinct race of apes, intermediate between anthropoids and man". Nowadays, though chance and perspicacity still count in the search for hominid fossils, the scene has shifted from South to East Africa. The size of the cast has increased and both the extent of backstage support and the scale of the budget reflect the fact that hominid research has changed a good deal in the past half century. Bernard Wood reports.

THE child's skull which Dart called *Australopithecus africanus*, the southern ape, had been discovered by accident (*Nature*, 115, 195; 1925). It had been found in the rubble of a lime quarry at Taung, which is about 80 miles north of Kimberley in what was then Bechuanaland. It was fortunate that the remains of a fossil monkey had been recognised at the same quarry only a few months previously. The child's skull was in fact identified, and then passed on to Dart, by Professor Young, a geologist and one of those who had been alerted that the site was of potential interest.

The Taung child was not the first fossil hominid to be discovered; evidence had been found in the previous century at several sites in Europe and by Dubois in Indonesia. The Taung child was, however, much less 'man-like' than any of these other candidates for a relative or ancestor of man,

despite the fact that its young age tended to mask these non-human traits. Also it was the first specimen to be found in Africa, a continent that has since given up so much evidence to support Darwin's speculation that it was the cradle of mankind. In South Africa after Taung, Dart and Robert Broom, a noted palaeontologist, learnt of other cave sites in the dolomite of the Transvaal. Although no more hominid remains have been found at Taung, literally hundreds of specimens have been found at four other cave sites, and work has been resumed recently at three of these sites.

The other concentration of hominid fossil sites is in East Africa, and the circumstances could hardly be more different. The East African sites are all associated with the Rift Valley where hominid bones, and the evidence of hominid activity, are preserved in the silt of ancient lakes and streams, in-

stead of having been carried, washed or dropped into caves as in South Africa. The Rift Valley sites are only revealed to us now because earth movements have so distorted the landscape of several million years ago so that what were beds and shores of lakes are now thrust up and tilted so that they are exposed to erosion by water and wind.

An advantage of the location of these sites in sedimentary basins is that, with the help of palaeontologists and those skilled in palaeoenvironmental research, the life and landscape that the early hominids knew can be reconstructed. The intensity of diversification in contemporary animals and knowledge of the habitat are but two of the new dimensions that have been introduced into the interpretation of hominid remains. The stratigraphy of the sediments also enables the location of specific fossil finds within a large site to be placed in an order of occurrence. The tectonic activity that elevated and tilted the fossil sites was accompanied by volcanic eruptions, the products of which are suitable for isotope dating. These eruptions periodically covered the landscape and are now incorporated as layers of jam in the sedimentary sponge-cake. The dates of these strata provide bracketing ages for the fossils that lie in the intervening sediments. The history of the Earth's magnetic field can also be traced in the sediments which preserve the perturbations of the magnetic poles. When this information is combined with the results of the isotope dating techniques the East African sites can be fitted into an absolute time scale enabling them to be placed in a historical context, one with another, and also with dateable sites elsewhere.

The problems of organising the teams of research workers necessary for modern research into human origins, in inaccessible areas and often in a harsh climate, are probably no better illustrated than at East Rudolf in North Kenya.

This site, comprising sediments exposed in five main regions over an area of 2,000 square kilometres, was not found by accident. In July 1967 Richard Leakey was leading the Kenyan contingent of a multinational expedition that had been organised to explore the fossil potential of the Lower Omo Basin in southern Ethiopia. The area allotted to the Kenyan team turned out not to be as rich as that allocated to the French and US contingents who have since successfully jointly explored the exposed sediments known as the Shungura Formation on the west bank of the river. Leakey, however, realised that in flying from Nairobi up to the fossil sites in Ethiopia he had flown over similar looking exposures

some several hundred kilometres further south on the north-east shore of Lake Rudolf. So, following a short aerial reconnaissance in 1967, the first East Rudolf Expedition was mounted by Richard Leakey in 1968. It explored the area as far as it could and found enough vertebrate fossils, including four hominid specimens, to encourage the National Geographic Society (staunch supporters of Drs Louis and Mary Leakey in Olduvai Gorge) to support a more extensive field programme the following year. Their faith was rewarded by further hominid finds, among them a complete cranium, and stone artefacts that appeared to be eroding out of one of the volcanic layers. Dating evidence indicated that the fossils were being collected from sediments laid down between 1 and 4 million years ago, with the younger localities tending to be in the north of the area and the older ones to the south.

Leakey had now moved to the National Museum in Nairobi. The museum authorities recognised the scientific importance of the work and since then the field expeditions have been organised from the museum, which has provided vital technical assistance and considerable logistic support. Despite the fact that many of the scientists involved are from Europe and the USA, the essentially Kenyan identity and basis of the expedition has never been in doubt.

For the 1970 season Leakey sought the assistance of Professor Glynn Isaac from the University of California. With Leakey leading the field team and the two acting jointly as scientific coordinators, they recruited geologists and palaeontologists and, with the continued backing of the National Geographic Society and a generous grant from the National Science Foundation (NSF), they began a planned programme of prospecting, excavations and earth sciences field work. Specialists were recruited to examine the fossils, both hominid and non-hominid, and by this time the scale of work necessitated the establishment of a permanent field camp at East Rudolf at Koobi Fora.

Like the creatures it set out to study, the expedition underwent its own evolution. As the involvement of the participating teams became deeper, and the importance of the laboratory and experimental support that was necessary for the field work was recognised, the East Rudolf Research Project was formed. This consolidated the close relationship of the research with the National Museum, and by means of a research council provided the museum with a panel of scientists which could advise not only on how best the current research could be prosecuted, but which

would also help to formulate future research policy for the area.

Research teams now come from both the USA and Europe to participate alongside the scientific staff of the National Museum. Each team is grant aided to a greater or lesser degree. The NSF in America and the Royal Society, the University of London and the National Environmental Research Council (NERC) in the UK have all provided support for research workers from many disciplines.

The maintenance of research teams many hundreds of difficult miles from the nearest town calls, however, for considerable basic logistic support in addition to that provided for by the research grants. Drinking water is several hours drive away from the main camp and a lorry is used solely to fetch water and firewood. Another lorry plies the barely passable tracks and roads to and from Nairobi with petrol and supplies. The buildings in the permanent camp have to be provided and maintained in an almost constant gale. To supply and maintain contact with small camps spread out over an area of thousands of square kilometres an aeroplane has been purchased which has since proved itself invaluable. To keep the twelve vehicles and two boats that are used in the field in good order a small garage has been built and for the field season a mechanic is resident at the main camp at Koobi Fora.

The responsibility for finding the funds for all these basic facilities has been accepted since the outset by Richard Leakey. Much of this money has been raised in the USA, a good deal of it from proceeds of lectures which serve the dual function of informing people of the research work

and raising the necessary funds. Paradoxically it is this willingness of a scientist to ask the public directly for money, instead of receiving it anonymously from the people through government and research grants, that seems, to earn the displeasure of some fellow scientists.

It is now a fact that the discovery of a possible ancestor of man several million years old is newsworthy. It is apparently also vexing that Richard Leakey, like his father before him, has a knack of exploiting this news to the advantage of his fund raising, and moreover doing it with a style and self assurance that is normally only associated with unravellers of double helices.

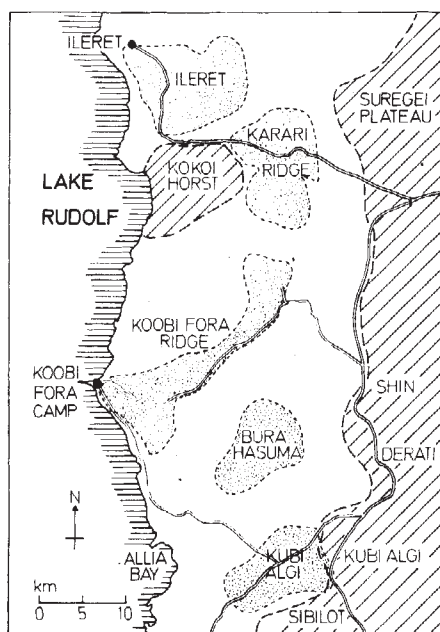
A more pertinent criticism of research programmes into human origins is that the very publicity surrounding the finds tends to concentrate judgement solely on results in terms of specimens recovered. There is an increasing tendency to judge sites according to their position in a hominid league table, with 'oldest' on one axis and 'greatest number' on the other. One would hope that, when scientific judgement is passed on these programmes, it will be on the basis of the quality of the scientific research and on the ability of the research workers to see beyond the immediate problems of the age and nature of the specimens. There is every sign that those bodies that support the research at East Rudolf appreciate the importance of the wider aspects of the research, while at the same time encouraging work on the fossils themselves.

Perhaps it is an encouraging sign of the maturity of hominid research that although research into the geological and palaeoenvironmental context of the material will be maintained at East Rudolf, and in some areas intensified, prospecting for hominid fossils has been temporarily suspended. This to give time for the sample of more than 120 hominids that have been collected to be assessed and analysed.

Only when the hominids are considered as just one faunal component, albeit an important one, of an evolving sedimentary basin, as is beginning to be the case at East Rudolf and at other East African sites, will hominid research this decade in Africa have shown itself to be a worthy descendant of Dart's pioneering efforts. That so much progress has been made, both in terms of available fossil material and in the manner in which its context is being studied, is to a large degree because of the scientific and organisational skills of two generations of the Leakey family.

A new centre for prehistory at Nairobi is to be called the Louis Leakey Memorial Institute. □

Fossil site, showing areas of exposed sediment



Transports of delight?



The two-car family on a half-acre plot in an isolated suburb is no longer considered ideal in Canada, where there is new thinking on the inter-relationship between transport and a better life. Angela Croome reports.

INNOVATIVE transport is at the heart of urban planning. If anyone doubts this it is not Canada's metropolitan authorities. In particular the richest and fastest growing province, Ontario, has put its money (half a billion Canadian dollars since 1971, and an estimated \$1.3 billion over a ten-year period) on this approach to achieving better living. Furthermore, there is confidence that by pioneering a new type of urban transit system and the urban planning philosophy on which it is based, Canadian concerns will soon be supplying most cities of America (North, South and Central) and will acquire for Canada a major new industry and sphere of expertise.

Last year more cars than babies were produced in Canada. At the same time the country is 'urbanising' at a rate of 4% a year, so that if present trends continue, 73% of the population will be concentrated in 12 metropolitan areas by the end of the century. A combination of the need for economies in the use of space and energy and an increasing dissatisfaction with suburban commuter living based on the car has endorsed a radical change in urban planning policy. This was initiated by the Ontario government as far back as 1969 but was underscored in 1971 by a now historic decision to cancel an urban thoroughway (for motor traffic) in favour of investment in better public

transport. In 1973 the Ontario government succeeded in pegging public city transport fares while improving services by offering local authorities massive subsidies to promote re-equipment and replacement (75% of cost) and reimbursing 50% of the losses on public transport operations. This move has already been reflected in increased public transport 'ridership' figures in most communities.

The problem is seen as "planning better communities with built-in mobility and closer jobs for everyone". "The two-car family on a half-acre plot in an isolated suburb is seen less and less as the good life". Thus says the eloquent and busy F. W. Foley, President of the Urban Transportation Development Corporation based on Toronto. Mr Foley spends much of his time putting across the new thinking on the inter-relationship between transport and a better life in cities. Toronto, the provincial capital, is the focus of these experimental developments. This is appropriate enough, as it has the reputation of being one of the most attractive and best-run of large American cities—and it is at the centre of a region of almost frighteningly rapid growth. It could get itself into the kind of jam that has long since engulfed New York and may be sampled on a smaller scale on the London-Essex margin of the Thames.

Toronto is set to be the demonstration city where the new approach can be tested in practice and where people may come and see for themselves. (The need to keep public reaction in step at every stage is wisely not lost sight of.) Already it has introduced a dial-a-minibus service in outlying districts

Krauss-Maffei TUO-3: withdrawn

where the travelling public is too small to justify a fixed-route service or an underground rail connection. Staggered working hours have already been introduced in city centre offices with excellent results. For some time a city task force has been taking an overview of Toronto's urban planning and transport situation, and involving the public in decision-making. The large Exhibition Park on the city's lakeshore has been picked for the demonstration of the unconventional intermediate transit system labelled "GO-urban" that is at the centre of the planners' thinking. With a track circling the park it will provide links for visitors between the various exhibition halls while at the same time testing elevated track, in-tunnel operations, driverless running, computer control, 'ride' comfort and the public reaction to all these elements.

The most significant step, however, has been the recent establishment with a Toronto headquarters of the Ontario Urban Transportation Development Corporation (UTDC) with a national and international remit. It is a body without parallel in Canada, and perhaps anywhere else. It is a business institution created by special Act of the Ontario legislature enabling provincial investment to be made in research, design, development and production of public transport systems on a commercial basis. Its role combines that of a think-tank in the transport field with an investment and licensing business somewhat analogous to Britain's National Research and Development Corporation (NRDC). Its immediate object is, simply, to produce "new

transit technology". Provincial Ontario's investment in transport equipment in the next decade alone is forecast officially at over \$1,000 million Canadian dollars; it is already spending nearly \$10 million a year on technology and operational research in this field. The federal government in Ottawa is watching the Ontario initiative and its UTDC with close attention. The province of Alberta has already invested in the corporation.

The key element in the transformation of urban living that the Ontario authorities seek to achieve is the intermediate transit system, using unconventional design. Broadly the object is to obtain the speed and accessibility of an underground (subway) system, but in an overground context with the greater comfort associated with medium density travel—20,000 to 30,000 people in each direction each hour—without the environmental blemishes of noise, fumes and massive land use, and at a fraction of the cost per mile of tunnelling or motorway construction. A world-wide investigation of the state of the art in rapid transit development was completed in 1972 and eight systems were identified for follow-up.

The Krauss-Maffei "Transurban" and Hawker-Siddeley Canada concept (with collaboration from two British companies) were taken up as detailed projects and the Krauss-Maffei system was chosen in Spring 1973. A kilometre-long test track with curves and gradients was completed at the Krauss-Maffei facility at Munich while preliminary site work was started at the Exhibition Park in Toronto. The investment was worth \$30 million but with tough 'break' conditions.

In parallel with these moves by the province of Ontario, Ottawa's Federal Ministry of Industry, Trade and Commerce secured the seven-man linear motor design team from Cambridge, UK, when Tracked Hovercraft Ltd (one of the firms originally associated with Hawker-Siddeley Canada's project) was closed down by the British government. They went to work for the Toronto company SPAR, specialising in advanced technology and space vehicle systems, and the federal government has made a \$2 million industrial development grant to it for work on linear induction motors. SPAR was obviously going to be the major contender for the motor contract for GO-urban propulsion when things got that far. A test track for running a motor palette in various configurations up to 70 miles an hour is now ready at SPAR's Toronto site. This initiative well illustrates the fundamental approach of the Canadian authorities—a deliberate and massive effort to absorb and vitalise a new technology and make it a national asset.

A back door into the supposedly lush US market for unconventional rapid transit systems (its urban and automobile problems being similar) opened last autumn when the giant McDonnell Douglas aerospace company of California announced a licence from Krauss-Maffei to market the GO-Urban system in the USA and its territories, and a collaborative agreement with Ontario's UTDC to participate in the technology and development of the Toronto demonstrator.

Very recently the line-up of interests and participants has changed fairly radically with the announcement that Krauss-Maffei was pulling out of the Toronto GO-urban demonstrator project.

Under the cancellation terms Krauss-Maffei paid \$1,800 million immediate cash compensation to Ontario's UTDC, which in addition has the use of the Munich test track and a number of German technicians for two years, together with outright transfer of the accumulated technology, a royalty-free licence for Canada and a non-extensive licence for the rest of the world.

If anything the withdrawal of Krauss-Maffei has put the Canadians in an even stronger position astride the unconventional rapid transit business at least in North America. A question that British experts have been asking for many months is why did the assessors for the Toronto demonstrator plump for a maglev system. The pause may provide just the opportunity to

move to another combination of drive and suspension while reserving rights in maglev technology for other applications. At the speeds envisaged (up to 50 miles an hour) rubber-wheeled carriages propelled by linear induction motors would be equally quiet and non-polluting. They would also make less demands in electricity (for the levitating magnets have a separate supply). It is noteworthy that a combination of advanced linear motor propulsion with rubber wheel guidance and suspension is the type of palette that SPAR, focally placed in Toronto, is currently concentrating on.

The province of Ontario is spending far more on redesigning its public transport than is being spent nationally anywhere else. Its fundamental 'systems approach' must be admired, if not copied. It will provide a blueprint for other industrial countries with growing urban problems. Whether the pre-occupation with new technology to solve the intermediate capacity urban rapid transit problem will be justified remains to be seen; certainly there is no existing vehicle or system that fulfils the specifications set out. There appears little likelihood of lifting the urban scene out of the doldrums and freeing the citizen from the predominance of the car without dedicated central thinking and action, and here Ontario and Toronto in particular have done the world a service and growing cities everywhere would do well to study these developments. □

Eight developers of promising systems representing a range of new technologies were invited by the Ontario Transport Ministry to make technical submissions. The eight systems provide a good indication of world-wide trends in the use and application of new surface transport technology. Only a Japanese submission is lacking—the Japanese have concentrated their effort and investment on high-speed inter-city links.

System Name	Design concept	Automatic command/control	Suspension	Propulsion
Alden "StaRRcar" (USA)	PRT*	Yes	Rubber tyres	Rotary a.c. motors, hydrostatic drive
Ford "ACT"	Line-haul or PRT	Yes	Rubber tyres	Rotary d.c. motors
Transportation Technology inc. (USA)	PRT	Yes	Air cushion	Linear induction motors
Uniflo (USA)	PRT	Yes	Air cushion	Linear air turbine
Bertin "Aerotrain" (France)	Line-haul	Optional	Air cushion	Rotary or linear induction motors
Urba "30/100" (France)	Line-haul	Optional	Negative-pressure air cushion	Linear induction motors
Hawker-Siddeley Canada (Canada)	Line-haul with off-line stations	Optional	Rubber tyres	Linear induction motors
Krauss-Maffei "Transurban" (Germany)	Line-haul or PRT	Yes	Electro-magnetic	Linear induction motors

* Personal rapid transit.

international news

AN unusually volatile mixture of politics and technology surfaced in the Commons in the first week of February. Its implications for the Canadian federal government's energy policy were serious enough, but because the case potentially pitted the strongly independence-prone Quebec government against an equally strongly federalist-minded Government of Canada, the outcome will be watched with great interest here.

In question was a deal Quebec officials had been quietly negotiating with representatives of France for construction in Quebec of a uranium enrichment plant to be known as CANADIF. This plant would cost from \$3,500 million to \$4,000 million. Because it would use the gaseous diffusion technique, it would require large amounts of electrical power (some 2,500 MW), and this power would be obtained from the James Bay development, an enormous hydroelectric project that itself has been a centre of controversy for some time. (Critics questioned how much of the James Bay power would be used for Canada and how much exported to the United States, and the Indians who lived in the wilderness that had to be flooded at first adamantly opposed the destruction of their hunting ground, then finally agreed after extended legal battles to accept payment of \$150 million and other concessions.)

In order to provide this power, extra capacity would have to be built into the James Bay station at a capital cost of \$1,500 million–\$2,000 million (roughly half the cost of the entire enrichment project). Even then, the enrichment plant would end up using a quarter of the hydroelectricity generated by James Bay—and the cheapest quarter at that. The uranium enrichment project would be used in part to exploit large uranium deposits owned by French companies in western Canada. But the enriched uranium would not be used in Canada, because Canada's own nuclear power system, CANDU, uses natural uranium fuel, unlike the French system. Furthermore, the export of enriched uranium would be used in the reactors of competitors to the CANDU system—perhaps not just the French, but the Americans as well.

All this at a time when Canada has restricted, and proposes eventually to eliminate, the export of energy in the

CANADIF raises energy policy problems

from David Spurgeon, Ottawa



Trudeau: no competition wanted

form of oil from Alberta and Saskatchewan to the United States—and at a time when Canada sees her own capital needs for energy as being approximately \$107,000 million in the next decade and when she has recently become so concerned about the use of her nuclear materials abroad that she has toughened up safeguard provisions at the risk of restricting her own CANDU sales.

The case came to light publicly when a number of documents showing the federal government's concern about the project were leaked to the Press, and Prime Minister Trudeau told the Commons that Canada is not interested in having enriched uranium exported in competition with the CANDU system. He said he had told France and Quebec about this disinclination, but he added that the federal government would still consider the deal if France, for example, wanted to build the plant itself, and if convincing arguments could be presented showing the scheme was in Canada's interests.

Commented *The Globe and Mail* of Toronto in its leader the next day: "It is difficult to see how the uranium enrichment plant proposed for Quebec would significantly enrich anybody except private industry, France and possibly some other foreign buyers."

One aspect that particularly worried critics of the scheme was that the consortium proposing it intended to amortise the cost over a period of only seven to eight years, and to give it a market lifetime of as little as 15 years, despite the fact that its technical lifetime could be 30 to 40 years. This seemed to indicate that they were hedging bets against the much cheaper centrifuge system of enrichment now being pursued by other European interests.

"Is Canada to be used as a colony, to provide supplies for the meantime, and then to be left with an antiquated plant on its hands?", asked *The Globe and Mail*.

The leaked documents showed how worried the federal government was that the Quebec government was trying to put the deal through quickly and quietly, thus presenting Ottawa with a *fait accompli*. This was not the first example of Quebec's attempts to establish a freedom of action usually associated with a sovereign state, and it was not the first example of France's desire to strengthen its influence and ties with francophile Quebec.

One of the leaked documents was a letter from the Federal Energy Minister, Donald Macdonald, to Mr Trudeau. He described the consortium proposing the plant as consisting of Canadian Pacific Investments–Cominco (20%), Commissariat à l'Energie Atomique (40%), and the James Bay Development Corporation (40%). He said the consortium seemed to think the federal government's concern was solely with nuclear safeguards.

"The prospect that the federal government might withhold licensing of such a project because of economic considerations had never been contemplated." To show the importance of these considerations, Mr Macdonald attached a memorandum from his deputy minister, T. K. Shoyama, which noted:

"(1) The federal government would have considerable concern over a large commitment towards an energy source to be utilised for the development of a product destined entirely for the export market.

(2) If there were a shortage of resources in the next decade, the government would want the available ones to be devoted to projects urgently required for the domestic economy.

(3) For a project to be deemed in the

national interest it would have to be seen to be paying at least the average industrial power rate in the province, not the incremental cost of new power generated by Hydro Quebec, which is expected to be lower.

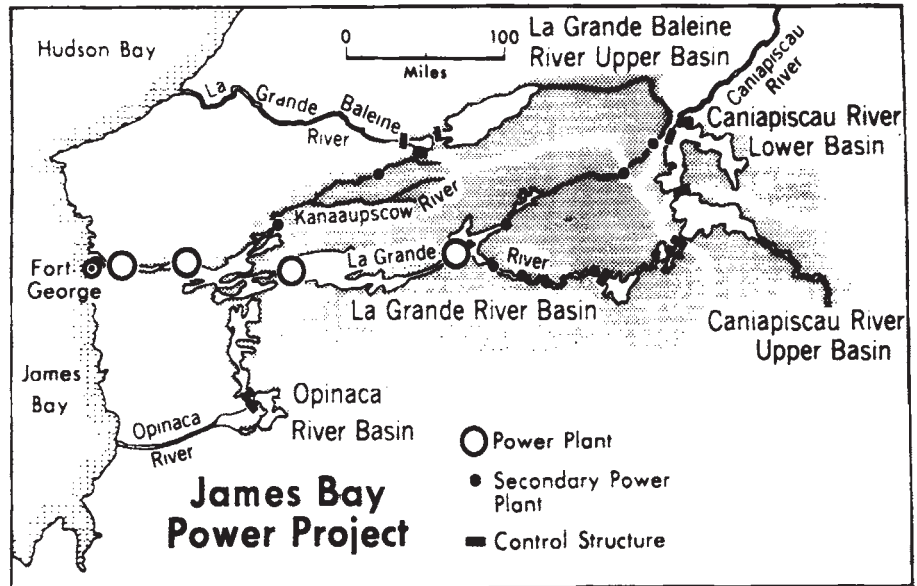
(4) CANADIF proponents wanted contracts for enriched uranium not to be interfered with for the life of the contracts, but 'Canada's safeguards policy could not be subservient to commercial considerations'. A country that might be an acceptable purchaser might later be found to be an unacceptable safeguards risk. If that proved to be the case, then the contract would have to be interfered with."

The memorandum made it clear that the federal officials feared Quebec's Premier Bourassa might try to enter into some commitment with the French government during a meeting he planned in December, and they warned Mr Trudeau and suggested he might speak to Mr Bourassa before that time.

Otherwise "it would be that much more awkward in the future for the federal government to refuse a construction permit through the licensing procedures of the Atomic Energy Control Board".

After his statement in the Commons, Mr Trudeau noted that even if non-Canadian uranium were used for enrichment and export by CANADIF, the federal government would remain concerned because large quantities of Canadian electricity would be required.

The irony of the situation is increased



when one recalls how proud Canadian atomic energy officials have always been in the past of the fact that the CANDU system itself does not need enriched uranium as fuel. For many years they had pointed out that the United States went along the enriched uranium route because she already had a massive investment in diffusion plants for nuclear weapons, while Canada, with no such plants, developed CANDU to exploit her abundant uranium deposits.

Until now, the Americans have been much more successful at selling their reactors abroad than have the Canad-

ions. Now there is the prospect of France exploiting Canadian uranium with the aid of Canadian electrical power in a period of power shortages, and selling it abroad not only to countries that may buy France's reactors but also to the United States.

By the end of the week, Premier Bourassa's only response had been that the CANDU reactor could be modified to use enriched uranium fuel. This is true, but it does not answer the question, why this should be done when CANDU was designed specifically so that it would not need enriched uranium. □

THE company formed to extract oil from the Athabasca tar sands in Alberta, Syncrude Canada Limited, has been saved by an injection of funds from the Canadian, Ontario and Alberta governments. The three announced they would invest \$600 million in the project in exchange for a direct share interest.

Until the announcement, the fate of the project—which aims to tap Canada's greatest potential source of hydrocarbon deposits—was in doubt. Last December, Atlantic Richfield Company of Los Angeles dropped its 30% interest because of mounting costs, currently estimated at \$2,000 million compared with the original estimate of \$195 million in the early 1960s. Shell Canada, which had shown an interest in joining Syncrude, later decided not to.

The remaining participants (Imperial Oil Limited, Gulf Oil Canada Limited, and Cities Service Company of Tulsa, Oklahoma) said they would abandon the project if they could not find additional partners.

So far, about \$160 million has been spent on the scheme. Syncrude scoops up the oil-saturated sand and then

separates the oil by means of hot water and steam. The site is near Fort McMurray, 225 miles north-east of Edmonton.

In return for its investment of \$300 million, the federal government will receive a 15% interest in Syncrude. Alberta gets a 10% interest for \$200 million, and Ontario 5% for \$100 million. Thus, governments will hold a 30% interest and private industry 70%.

Energy critics attacked the federal government's decision in the Commons after the announcement. T. C. Douglas, of the New Democratic Party, said "it represents the greatest sell-out of our natural resources in the history of Canada." He claimed it would benefit no one but the oil companies involved. And there is no way the government can avoid extending similar benefits to other companies, he said. Mr Douglas thought the government, together with other provincial governments that wanted to participate, should take over the project entirely.

Ontario's Premier William Davis sees it differently. He compared his province's investment to the gamble that was taken 100 years ago by those

who invested in the first railways across Canada. But Saskatchewan's Premier, Allan Blakeney, said that the federal government's decision to allow the Syncrude consortium to charge higher oil prices and greater tax deductions for royalties paid to the Alberta government is discriminatory, because his province's oil producers' prices are frozen and they cannot deduct royalties.

Subsequent questioning in the Commons made it apparent that the federal government's investment could climb higher than the \$300 million first promised. The Treasury Board President, Jean Chretien, said Ottawa's commitment is fixed at 15% of the costs, without an upper limit.

But it was not clear how high the investment would climb without question. Any major change in costs, Mr Chretien told reporters, would mean that "all the parties involved will have to sit down and review the situation."

It was also revealed later that Alberta's government will lend Gulf Oil and Canada Cities Service Limited \$100 million each, and will spend \$500 million—\$600 million on a power plant, pipeline and municipal services for the project.

Soviet biologist arrested in Moscow

from Vera Rich

THE arrest of the Moscow biologist Sergei Kovalev on December 27, 1974, seems to mark yet another hardening of Soviet pressure on dissident intellectuals.

Kovalev, whose main scientific work has been in the fields of mathematical biology and genetics (itself, until recently, a suspicious activity, by Soviet standards) is a member of Sakharov's Initiative Group for the Defence of Human Rights in the USSR, and also of a small group of Amnesty International, set up in Moscow in 1974. He has been active in the defence of prisoners of conscience within the USSR, and in campaigning for the free circulation of ideas and information. In 1974, when the *samizdat* "Chronicle of Current Events" resumed publication after an 18 month gap, Kovalev was one of the three dissidents who publicly claimed responsibility for its publication, being convinced "of the necessity for truthful information concerning the infringements of fundamental human rights in the USSR and for its availability to those interested." On the very day of his arrest, in a New Year's message telephoned to London, Kovalev and Sakharov appealed "from a huge and tragic country, the fate of which has huge influence on the life of the whole world" for a general amnesty for prisoners of conscience, mentioning among others mathematician Leonid Plyushch, biologist Vladimir Bukovskii, and psychiatrist Semeon Gluzman. "Let a general political amnesty open for our country and for all countries of the world the way to freedom and a good and sensible life."

The same day, Kovalev was arrested,

apparently on a charge of being in contact with the illegal *Chronicle of the Lithuanian Catholic Church*. This journal, which has a nationalist as well as a religious slant, has already been the cause of arrest of a number of Lithuanians; at the end of November, however, at the request of the Lithuanian KGB, a number of prominent Moscow dissidents, including physicist Andrei Tverdokhlebov, had their apartments searched. Like Kovalev himself, those involved do not appear to be Lithuanians, Kovalev's professional contacts with Lithuanians appear to be slight—only one of his published papers (*Biofizika*, 15, No. 1, 147–155) includes a Lithuanian, I. Dudzyavichyus, among the co-authors. He is, however, reported to have intervened in April 1974, in the case of a Lithuanian woman claiming US citizenship, who was arrested while attempting to reach the American Embassy in Moscow. It would seem difficult, simply on these grounds, to present Kovalev as a fanatic in the cause of Lithuanian separatism.

Nevertheless, the charge is that of possessing and disseminating copies of the *Chronicle of the Lithuanian Catholic Church*. Whether in fact Kovalev did, in his capacity as a campaigner for human rights, have some copies of this Chronicle in his possession is not known. The accusation does, however, have a number of advantages for the prosecution. Firstly, the case against the *Chronicle of the Lithuanian Catholic Church* has already been opened, and it is simply a matter of adding another name to the list of the accused, rather than opening a new case. Second, the trial will take place in Vil'nyus, capital of the Lithuanian SSR, away from the focus of attention which a Moscow trial would be for Western journalists. And thirdly, in a society

which extols atheism as "scientific", the attempt to implicate Kovalev with a religious journal may be, to a certain extent, a kind of smearing of his intellectual status.

Nevertheless, these moves to dispel publicity from the arrest and forthcoming trial have not met with unmitigated success. An appeal organised by Academician Sakharov on Kovalev's behalf has been signed by no less than 50 Soviet intellectuals, including linguists, mathematicians and research scientists. □

Toxic chemicals register first step

from Peter Collins, Geneva

BASIC agreement on the establishment and organisation of the proposed International Register of Potentially Toxic Chemicals (IRPTC), was reached at the meeting held at Bilthoven, Netherlands, last month (*Nature*, January 17). Interest in the meeting was considerably more than had been expected by its organisers, the Netherlands government and the United Nations Environment Programme, 57 scientists representing 27 countries and institutions being present. Besides the decision on basic organisation (a central unit correlating and disseminating data from a network of national sources) there was fruitful discussion of the type of work programme that could realistically be undertaken in compiling the register.

For this purpose, there should be an "intensive" programme, providing comprehensive information on a relatively small number of particularly important substances; and a much larger "extensive" programme listing a restricted number of attributes of many more compounds. The intensive programme, it is suggested, should start with a pilot project confined to a small number of chemicals; the object would be to test the best format for presentation of the material, to study means of collecting the data and checking their validity, and to estimate the costs of operating this programme. The extensive programme, on the other hand, would be "open-ended", and might start by making use of information from existing data files. Use could be made of the facilities of the UNEP's International Referral System for Sources of Environmental Information (IRS), which is in a position to provide information on the whereabouts and capabilities of existing data collections. This initial survey should cover information of a fairly general nature as well as data on individual chemicals. It should be matched by a survey of potential users of the register, and could thus lead to a preliminary version of the proposed network directory which

"CHEMOSYNTHESIS", the reduction of metals from their ores by means of bacteria, is becoming an important field of research in the Soviet metallurgical industry, and is considered of great potential in the use of a wide range of ores from bauxite to gold agglomerates and uranium ores. Their use of bacteria ranges from the effective utilisation of ores such as the carbonate ores of manganese, where present methods lose up to 35% of the available metal (the chemosynthesis method yields up to 97.5% metal even from low-grade ores), to the extraction of gold and tin from arsenic-bearing agglomerates.

While some such processes are still only in the pilot-plant stage (the extraction of gold, manganese, bismuth, lead, antimony, lithium, or germanium) or

else exist only as theoretical projects (extraction of nickel, thallium, molybdenum, titanium and the microbiological lysis of aluminosilicates), some have already been introduced on a small industrial scale. Such plants, for the extraction of copper, uranium and zinc, are already operating in Kazakhstan and the Urals.

An intensive search is going on, in the research institutes of the Ministries concerned, to find new strains of thermophilic bacteria, multiplying at 55–60° C, which can reduce metal ores. The details of the processes involved remain obscure, but investigations on gold ores have given strong indications that the bacteria and other micro-organisms concerned are capable of forming organic compounds containing the gold in the course of their metabolic processes.

EXPENDITURE by the Department of Industry on research and development has risen 18% to £83 million in the year 1974/5 (*Report on Research and Development 1974*, HMSO £1.10). This barely keeps pace with inflation—even less so when allowance is made for £1 million or so of Rothschild-type transfers to the department in the last year. And when the applications and satellites (expenditure on which, in the form of subventions to the European Space Research Organisation, has risen in a year from £6.9 million to £15.4 million) are removed, the growth is decidedly negative.

Departmental expenditure on exploration and exploitation of the Earth has almost doubled in real terms in the last year to £5.5 million, all the new money going on the marine side. On the other hand civil aeronautics (excluding Concorde), hardly a booming business at present, declines 15% in real terms, getting £17 million of support.

Industry and government establishments now almost equally divide the department's money, industry having increased its share from 42% to 49%. The department's largest laboratories, however, the National Physical Laboratory and the National Engineering Laboratory suffer 10% cuts in real terms.

● Dr Walter Marshall, Chief Scientist at the Department of Energy, was given a rough ride last week by the Select Committee on Science and Technology who were clearly astonished at the lack of progress in formulating a strategy for Britain's research and development into means of energy conservation and developing new energy sources.

The Department of Energy is "still in a thinking period," Dr Marshall told the select committee when asked about the achievements of the multifarious committees that are currently worrying about energy. Dr Marshall and other members of the Advisory Council for Research and Development of Fuel and Power (ACORD) which reviews the research and development of the nationalised energy industries were closely questioned about the specific achievements of this body amongst others.

Dr Marshall said that he expected to produce a strategy in the next few months but that he did not like making promises. The matter of energy research was extremely complex and it had to be recognised that in energy research, the time scales between research and commercial application could be decades.

He was certainly disappointed that the level of spending on energy (other than nuclear power) remained at the present level, and the energy pro-

Round Britain

gramme will demand more money over the years. In the short term, Dr Marshall admitted that conservation schemes would be given priority over schemes to develop new sources of energy as they produced the most immediate savings. He defended the seeming lack of progress since his appointment six months ago by telling the committee that although it was necessary to get going on the energy programme quickly, it must be in the right direction, which meant a great deal of preparatory work.

● Before last year's somewhat abortive International Law of the Sea conference at Caracas, the responsible British minister at the Foreign and Commonwealth Office, David Ennals, held a day-long meeting in London which all interested parties could attend, ride their hobby horses and tilt at their favourite windmills. The Conference reconvenes in Geneva in March with a heightened need for agreements and a convention. On January 30, David Ennals chaired a second get-together at Church House but the level of argument seemed to have been pulled down by the overlong and over-strident proceedings at the international level at Caracas. A disproportionate time was spent on airing the narrow prejudices of the fishing industry presented as facts. The problem of enforcing penalties for oil pollution at sea seemed as far away as ever though a possibly practical suggestion was to invoke the responsibility of the port of origin as

well as the supposed flag of the offending vessel — too often one of convenience.

● The future of the Climatic Research Unit at the University of East Anglia now seems assured. After a period in which the work of the unit was severely hampered by uncertainty about where funds would be coming from over the next quinquennium, the Director, Professor Hubert Lamb, has been able to announce the award of several major grants to his team.

Largest of these is £100,000 from the Wolfson Foundation, covering the years up to June 1979 and "with no strings attached", says Professor Lamb. A Rockefeller Foundation grant of \$120,000 is specifically for a project on mapping and analysing available reports of weather, chiefly from various parts of Europe and Iceland, which cover many centuries in the past. This project will run until October 1977, and it is hoped to extend the analyses back for 1,000 years.

Professor Lamb has stressed the urgent desirability of increasing the number of staff in his unit, and a Nuffield Foundation grant of £24,228 will provide for the appointment of a Deputy Director from March 1976 to December 1979. The unit is also carrying out work under contract for various interested parties, including the City Authorities of Hamburg, who are interested in the incidence of disastrous storm floods in the North Sea, and the Commercial Union Assurance Company, which is supporting a three-year study of changes in the global incidence of tropical cyclones.

The dramatic turnaround in the unit's fortunes is also highlighted by Professor Lamb's future plans. His ambition for the unit to become more interdisciplinary in nature seems likely to be fulfilled, with a Nature Conservancy Council contract for a biologist to study effects attributable to climatic change on the distribution of the natural flora and fauna of the UK and North-west Europe, and "hopes of appointing an economist to keep watch on the impact of current climatic fluctuations on the world food situation, trade and other international aspects".

would be one of the main preoccupations of the central unit.

For positive identification of compounds included in any part of the register, use would be made of the Chemical Abstracts Service (CAS) Registry Number, which is already used by most data collections, although there will certainly be many instances in which this cannot be used.

A major problem may be the coverage of trivial and trade names of substances in languages other than English,

and it is here that international co-operation, especially with manufacturers, will be most necessary.

Consideration was also given to the hardware and software that will be required as the register is developed. One facility that could be of great use is the International Computing Centre, a UN inter-agency facility in Geneva, but it is appreciated that not all storage of data will be computerised, especially in view of the marked interest in the register already being shown in some of the

developing countries.

The presence of scientists from the Soviet Union and Hungary, as well as from Brazil, Ghana, Togo, the Philippines, Tanzania and India, in addition to almost all the highly industrialised countries, shows that the need for this new move in environmental protection is widely appreciated.

With a bit of luck the work of the group will not be hamstrung by the economies being applied elsewhere in the United Nations system. □

A COMMITTEE of the National Academy of Sciences last week shook the foundations of energy planning in the United States by suggesting that domestic supplies of oil and gas may dry up in 25 to 30 years, much sooner than the government has been forecasting. If the committee's estimates turn out to be correct, deep trouble is in store for the much-vaunted drive to make the United States less dependent on imported oil to meet its energy needs.

The estimates form part of a 2-year review of minerals supply and demand, conducted by the academy's Committee on Minerals Resources and the Environment (COMRATE). The committee's central, and inescapable, conclusion is that much more vigorous conservation efforts are needed, not only for oil and gas, but for various other materials as well. As COMRATE's chairman, Dr Brian J. Skinner, professor of geology and geophysics at Yale University, put it last week, conservation should become "almost a religion" if serious dislocations are to be avoided.

Although there is no single "official" estimate of oil and gas resources in the United States, those most commonly used for government planning have been developed by scientists working for the US Geological Survey (USGS). The USGS figures generally suggest that domestic production of oil and gas can be expanded over the next decade, and that supplies will be good for at least 40 or 50 years.

But those forecasts have been challenged recently by much of the oil industry and a number of distinguished experts. They have argued that the USGS's estimates are grossly overblown and that domestic resources are already beginning to dry up. COMRATE's entry into the fray last week with an estimate that broadly supports USGS's critics is likely to sharpen the debate considerably.

The importance of the dispute is this. If the USGS's figures are correct, expansion of oil and gas production in the United States over the next decade or so will be a great help in alleviating dependence on Arab oil. Projections developed last year by the Federal Energy Administration, for example, envisaged domestic oil and gas production going up by more than 50% by 1985, given the economic incentive of high oil prices and some governmental support. But if the USGS's critics are correct, expanded domestic production can be virtually ruled out. Moreover, there will be an acute need to develop alternative fuels (such as synthetic petroleum and gas from coal, and oil from shale) as quickly as possible.

There is virtually no dispute about the size of the so-called "proven" reserves—those which the oil industry

Bad news for US energy planners

by Colin Norman, Washington

has already found and believes it can exploit commercially with conventional technology. The nub of the disagreement concerns the extent of oil and gas deposits which have yet to be discovered—the so-called undiscovered recoverable resources.

In March last year, USGS Director Vincent McKelvey published a set of figures suggesting that undiscovered oil resources in the United States and offshore amount to between 200 and 400 billion barrels, while undiscovered natural gas resources total between 990 and 2,000 trillion cubic feet. By comparison, estimates developed by two oil companies last year put the oil reserves at about 90 billion barrels and gas reserves at about 400 trillion cubic feet. Now COMRATE has come up with a forecast that about 113 billion barrels of oil and 530 trillion cubic feet of gas remain to be discovered.

Surprisingly, those estimates differ little on the extent of oil and gas resources in Alaska and offshore, although COMRATE estimates that fully 70% of future supplies will come from those areas. The chief discrepancy is to be found in the forecasts for the extent of undiscovered deposits onshore in the lower 48 states, and that fact is surprising because the continental United States is about the most extensively drilled area in the world.

The difference is explained by the fact that there are two schools of thought about how the resources should be measured. The oil industry says that the USGS estimates fail to take sufficiently into account the trend of declining oil production per foot of drilling over the past few years, while the USGS says that the oil industry's estimates pay insufficient attention to potential production from small fields.

Until recently, USGS's figures were based essentially on a method which involves extrapolating production rates for oil and gas from known fields to similar geological deposits elsewhere. But that methodology has come under increasing attack, most prominently by M. King Hubbert, a geologist who used to work for Shell, but who now works for the USGS. Hubbert maintained, as long ago as the 1950s, that such predictions are based on production data from the richest parts of existing fields, and thus fail to take into account the fact that the amount of oil produced per well in a given field declines with the number of wells drilled. In other words, the oil com-

panies have creamed off the most productive areas of Texas, Oklahoma and California and information from those areas is unreliable as a guide to what is likely to be found elsewhere.

The USGS's more recent estimates have gone some way towards meeting Hubbert's objections, since they assume that unexplored areas will be only about half as productive as areas which have already been exploited. But Hubbert argues that even those revised estimates will prove to be much too optimistic. A better estimate, he says, is that they will be about one tenth as productive.

Hubbert has at least one important piece of evidence in his favour. In the mid 1950s, he predicted that domestic oil production in the United States would peak in the late 1960s and thereafter would decline; the USGS was then estimating that the peak would not come until the mid-1980s at least.

As it turned out, the peak came in 1970. Hubbert's critics point out that environmental constraints, coupled with a moratorium on offshore oil drilling, may have been responsible for some of the decline since 1970, but Hubbert's arguments have steadily gained acceptance in the oil industry, and in particular they have forcefully been taken up by John Moody, a former official of Mobil Oil and now a consultant to the oil industry. Moody, who was a member of the COMRATE panel, estimated last year that undiscovered oil reserves amount to about 90 billion barrels.

For its part, COMRATE has come close to accepting the validity of Hubbert's approach. Its report states that the projections developed last year by McKelvey "could have been more rigorously derived" and COMRATE's estimate that undiscovered reserves amount to about 113 billion barrels is closer to Hubbert's prediction than to that of the USGS.

Asked what effect he expects the COMRATE report to have, Skinner said last week that "now that the National Research Council (the operating arm of the National Academy of Sciences) has put out a report that comes down in favour of the lower figures, not firmly but at least in favour of them, there will be a lot of pressure on the Geological Survey to justify its methods".

A USGS official noted, however, that oil resources have consistently been underestimated by the oil industry in the past. "Who is to say who is right?", he said, "We are not going to back off from those figures just because they are high". The USGS is, however, updating its estimates and hopes to have a new set of figures, based on the most recently available geophysical data, ready by April. □

news and views

Io, the anomaly of the Solar System

from a Correspondent

JUPITER is surrounded by an extensive system of 13 satellites. They include four large planetary bodies Io, Europa, Ganymede and Callisto whose sizes are similar to that of our Moon. These satellites reside in the region of trapped radiation around Jupiter and their interaction with the Jovian environment is much greater than the Moon's with the Earth's magneto tail. But it is Io that is the anomaly, not only among the Galilean Satellites but also among all the bodies of the Solar System.

The density of Io, 3.5 g cm^{-3} , is the highest of the Galilean Satellites and comparable to that of the Moon and Mars. In general high-density bodies ($\rho > 3$) are probably rocky silicate structures whereas low density objects ($\rho < 2$) may have a large percentage of ice. During the Pioneer 10/11 flybys, it was found that Io efficiently absorbed electrons in the range 0.16–9 MeV (Fillius and McIlwain, *J. geophys. Res.*, **79**, 3589; 1974; and Simpson *et al.* *J. geophys. Res.*, **79**, 3522; 1974) but had little effect upon electrons of higher energies. Earth-based radio observations had shown that the position of the satellite on its orbit influences the

decametric radio bursts from Jupiter.

Visual astronomers discovered a further puzzling feature. Some reported an increase in brightness of 0.1 mag lasting as long as 15 minutes upon re-emergence of the satellite from Jupiter's shadow though other observers have failed to detect any change in brightness. Does this suggest that the satellite may have an atmosphere? Certainly one would not expect a planetary body with such a low surface gravity to possess a substantial one, so that the discussions of an atmosphere on Io have always been controversial.

But Io does have an atmosphere and an ionosphere too—it is the smallest body in the Solar System with such features. The ionosphere was detected during the Pioneer 10 flyby (Kliore *et al.*, *Science*, **183**, 323; 1974) and was found to extend to some 700 km above the surface with a peak electron density of about $6 \times 10^4 \text{ electrons cm}^{-3}$ at an altitude of between 60 and 140 km on the dayside. A thinner and less dense region was observed on the nightside with a peak density of $9 \times 10^3 \text{ electrons cm}^{-3}$ at an altitude of 50 km.

The diminished nightside ionosphere indicates that this region is produced by the action of solar radiation on the dayside and then decays during the 21 hour Io night.

The Io atmosphere is tenuous, with exotic constituents sodium, calcium, hydrogen, possibly also ammonia and nitrogen, and a surface pressure of between 10^{-8} and 10^{-10} bar. Sodium emission from the satellite's atmosphere provided Brown and Chaffee (*Astrophys. J.*, **187**, L125; 1974) with the first positive evidence for the atmosphere. Trafton, Parkinson and Macy (*Astrophys. J.*, **190**, L85; 1974) reported that the emission came from an extended space around Io. The total column abundance of sodium is estimated to be roughly 10^{11} cm^{-2} in this extended cloud and 10^{13} cm^{-2} in the atmosphere of Io.

As well as the sodium cloud, there is around Jupiter in the orbital plane of Io an extensive cloud of atomic hydrogen (Carlson and Judge, *J. geophys. Res.*, **79**, 3623; 1974). The mean diameter of the torus is about equal to the diameter of the orbit of Io. The torus is not complete, however, but seems to

JOVIAN thunderbolts, occurring at a rate of one per square kilometre of Jupiter's surface every 10 min, may explain the presence of acetylene in the clouds of the giant planet.

Both ammonia and acetylene have been detected in Jupiter's atmosphere, and since both would be rapidly photodecomposed they must be being continuously produced by processes operating in the planet's atmosphere. According to Bar-Nun (*Icarus*, **24**, 86; 1975) ammonia could be produced in the hotter deep layers of the atmosphere and persist for long enough to be carried upwards by convection into the layers where it is detected. But the lifetime of acetylene under Jovian conditions is so short that it must be made closer to the top of the atmosphere.

Bar-Nun's model, which is backed up by laboratory experiments, suggests that the shock waves of thunderstorms are the chief mechanism by which acetylene is produced on



Great Red Spot plastic wrapped by Jovian bolts

by John Gribbin

Jupiter. The electric discharges of lightning will themselves contribute to some extent, but a much greater volume of gas is affected by the thunder,

with the result that significant quantities of methane are converted to acetylene. Ammonia will also be produced in this way, together with hydrogen cyanide, cyanogen and other compounds.

Taking a typical terrestrial storm as a guide, Bar-Nun calculates that $5.3 \times 10^4 \text{ km}^{-2} \text{ yr}^{-1}$ thunderbolts would be needed to produce the amount of acetylene detected. Since Jovian bolts are likely to be more impressive than their terrestrial counterparts, the number may in fact be rather smaller. The compounds produced in these shock waves are almost ideal for explaining the puzzle of Jupiter's colouration, with yellow-brown acetylene polymers and ruby red polymers containing hydrogen cyanide and cyanogen predominating; the correct colour intensity for the Great Red Spot could be produced if thunderstorm activity in the spot is larger than the Jovian average by an order of magnitude.

extend for 60° on either side of the satellite. The lack of emission from that portion of the cloud which happened to lie in Jupiter's shadow suggests that the cloud's diameter is smaller than that of Jupiter and that resonance scattering of sunlight is the primary excitation mechanism.

A torus of this type is thought to be formed by atoms which can escape from the satellite but do not possess sufficient energy to escape from the vicinity of the planet owing to its large gravitational field (McDonough and Brice, *Icarus*, **20**, 136; 1973). Consequently the atoms are bound in closed orbits until lost by ionisation or recapture and tend to produce a toroidal shaped cloud whose density is determined by the ionisation losses. The cloud around Jupiter has a brightness of about 10^4 Rayleighs, composed of roughly 10^{33} hydrogen atoms with a mean lifetime of $\sim 10^5$ seconds. This is slightly longer than Io's orbital period.

But is Io the source of the toroidal hydrogen? The escape of hydrogen from Io poses no problem, since the Jeans escape from an exosphere as cold as 200 K is still very efficient. In order to maintain the cloud the satellite must be supplying hydrogen at a rate of 10^{11} atoms $\text{cm}^{-2} \text{ s}^{-1}$. An escape flux of this magnitude would exhaust the entire atmosphere of Io in a few years. Is the surface of Io abundant in hydrogen-rich material?

There are several other puzzling questions regarding the surface of Io, a principal one being of course why it has such strong sodium emissions. Also, why is Io as bright as if it were covered by ice, yet shows no ice absorption features in its spectrum? And why has Io dark poles?

Fanale *et al.* (*Science*, **186**, 922; 1974) suggest that Io's surface composition involves evaporite salt deposits which are rich in sodium and sulphur. (This would both provide a source of sodium and explain the brightness of the planet.) They believe that unlike the other Jovian satellites, Io never had large amounts of ice, yet it apparently was not totally devoid of water like our Moon. As a result of internal degassing, much of Io's water may have seeped to the surface, where it would quickly evaporate as a result of the satellite's proximity to Jupiter and its low surface gravity. The high density of Io and absence of H_2O ice bands in the surface spectrum may mean that this process is essentially completed. Fanale *et al.* speculate that the internal processes are considerably less advanced on Europa and Ganymede, satellites which are apparently very different from Io.

Salt-rich assemblages that Fanale *et al.* propose for the surface of Io are easily derivable from the leaching of carbonaceous chondritic material.

Matson *et al.* (*Astrophys. J.*, **192**, L43; 1974) suggest that the sodium observed in the atmosphere is sputtered from Io's surface by the charged particle bombardment and these atoms could populate the cloud observed beyond the satellite. This mechanism may also be responsible for the calcium emission which Meckler and Eviatar (*Astrophys. J.*, **193**, L151; 1974) have observed at 4,427 Å. The previously unexplained colouration of the surface must therefore result from the Jovian magnetospheric fluxes being strongest at the poles of Io.

The presence of ammonia on the surface and in the atmosphere of Io is still controversial, but is required by McElroy *et al.* (*Astrophys. J.*, **187**, L127; 1974) to explain the strong sodium emission. Their hypothesis of the mechanism of sodium emission predicted the ionosphere we have now observed. They suggest that if Io's atmosphere contains $\sim 10^{18}$ molecules cm^{-2} of ammonia, its photolysis would lead to the production and escape of hydrogen at the rate of 2×10^{11} atoms $\text{cm}^{-2} \text{ s}^{-1}$. This process represents a net absorption of solar energy and heats the upper atmosphere to around 500 K. Nitrogen would be an important minor product, which when excited by an auroral mechanism could interact with sodium to cause sodium emission. McElroy *et al.* suggest that sodium photoionised by solar radiation may be the dominant source of ionisation. The aurorae possibly resulting in the

sodium emission may draw energy from the current arches to Jupiter, so that significant localised atmospheric heating may result, providing for thermal escape of exospheric sodium into the surrounding cloud.

The Io-controlled radio activity of Jupiter is highly sporadic so that a synoptic patrol of the brightness of the sodium emission may be used to examine the various roles suggested for the high energy particles. But Bergstrahl *et al.* (*Astrophys. J. Lett.*, in the press) found that the resonant scattering of sunlight was primarily responsible for the brightness of the sodium cloud. Auroral processes may still be important for the near surface emission and for heating sodium to its high kinetic temperature. The stability of the cloud over 43 nights of observation implies a remarkably steady source of sodium, since the mean lifetime of steady sodium is less than 16 days.

Io is an anomalous fragment of primordial material bathed in a violent electrical environment which modifies and erodes it. The Jupiter-Io system represents a unique environment where two atmospheres are mutually interacting on a large scale through a magnetic field (for example Goldreich and Lynden-Bell, *Astrophys. J.*, **156**, 56; 1969). Correlated ground-based observations, as well as *in situ* measurements from spacecraft are still required if we are to fully understand this strange planetary body and its interaction with the Jovian environment.

Cosmic rays from the Galaxy

from a Correspondent

THE origin of the high-energy cosmic ray particles ($\geq 10^{17}$ eV) remains a mystery. The long existing controversy on whether the particles observed at the Earth originate inside or outside the Galaxy seems however to be nearing resolution; evidence is accumulating in favour of Galactic sources.

The primary particles are difficult to detect because of their low flux $\sim 1 \text{ km}^{-2} \text{ yr}^{-1}$ at 10^{19} eV. Use is made of the atmosphere as a detecting medium by observing the extensive air showers (EAS) initiated by the primary particles. The secondary particles produced in one of these showers spread out laterally and can be picked up by an array of detectors over an area of several kilometres at sea level. The direction of motion of the original incoming particle can be estimated (typically with an accuracy of about 2°) by timing the sweep of the front of the

EAS across the detector array. By building up statistics over periods of years the distribution in arrival direction of the primaries can be determined.

There are several large EAS detector arrays situated around the world, most notably at Haverah Park, near Harrogate (England), Volcano Ranch (New Mexico), Yakutsk (USSR) and Narrabri near Sydney (Australia). Until very recently the combined results of all the arrays have failed to show up any evidence that was inconsistent with the complete isotropy of the arrival directions of these high energy particles. At lowish energies this is not too surprising as the magnetic fields in the Galaxy can churn up the particles so that on detection their direction of travel bears no resemblance to the direction of their original source.

Sufficient data on the EAS above 10^{19}

eV have now been accumulated, however, and these clearly indicate, for the first time, that there is some degree of anisotropy in arrival directions. By combining together data from the four arrays Krasilnikov *et al.* (*J. Phys.*, **A7**, L176; 1974) have analysed 76 events above 10^{18} eV arriving at the Earth from the northern celestial hemisphere. In an harmonic analysis in right ascension of the arrival directions of these particles a first harmonic amplitude of roughly 40% appears with a maximum occurring about 13 h RA. The authors calculate a chance probability of only $\sim 2.6\%$ for such an effect and add supporting evidence for it being real from data accumulated on lower energy EAS.

Such an anisotropy has obvious importance in connection with the origin of the particles. Hillas and Ouldrige (this issue of *Nature*, page 609) point out that this adds weight to the Galactic origin theories, supporting arguments based on other experimental data including the absence of a cut-off in the energy spectrum below 10^{20} eV due to 2.7 K photons. Plotting the available data on full celestial coordinates Hillas shows that the anisotropy in RA arises from a definite clustering of the arrival directions in at least three regions of the sky. Such separate clustering does not suggest a single distant source but rather a Galactic origin. With this in mind Hillas and Ouldrige further suggest, to explain the total cosmic ray energy spectrum, that the mode of propagation existing below 4×10^{15} eV, consistent with cosmic rays escaping along magnetic field lines, is overtaken at higher energies continuing right up to 10^{20} eV, by a more rapid drift, perhaps radially outwards across the field lines. This suggestion leads to an explanation of the position of the most prominent of the observed clusters. But as the authors point out, to retain nuclei at about 10^{20} eV (the highest energies so far observed) would require a magnetic field extending several kpc from the Galactic plane and even then with a field magnitude of about 2×10^{-6} gauss only relatively heavy nuclei could be contained. There is some other evidence from observations at radio frequencies of the presence of such an extended magnetic field.

Unfortunately it will take several more years of EAS observation to add significantly to the statistics of the anisotropy. Meanwhile at Haverah Park and elsewhere attention is strongly directed towards determining the mass of the primary particles in the energy range $\sim 10^{18}$ eV. Evidence in favour of a high proportion of multi-nucleon primaries at these energies would give good support to the Galactic theories.

Redshifts of BL Lac objects

from R. F. Carswell

BL LAC is the prototype for a class of astronomical objects which has been the subject of a good deal of observational and theoretical work over the past two or three years. Generally, objects of this type are notable for their rapid variations in intensity and polarisation at all wavelengths where these quantities can be observed, and for the absence of any discrete features in their spectra. Some, notably BL Lac itself and, for example, AP Lib and Markarian 501 seem to be surrounded by nebulosity and so look rather like galaxies with exceptionally bright nuclei, similar to Seyfert galaxies. Others, such as OJ287, are stellar in appearance and could be related to the quasi-stellar objects, though without any of the emission lines from which a redshift can be determined. In the case of such star-like continuous objects we can only conjecture at their distance from us, since no angular size or redshift can be measured, but for objects with nebulosity we can hope to obtain estimates of their distance by looking for observable features in the galaxy component.

Just such an observation has been performed in the case of AP Lib by Disney, Peterson and Rodgers (*Astrophysical Journal Letters*, **194**, L79; 1974) using a number of spectra taken at the Mount Stromlo Observatory in Australia. They noticed that a number of features could be seen very weakly but consistently on their plate material gathered over a period of time, and found that these correspond to lines often seen in normal galaxies if AP Lib has a redshift of $Z=0.0486$. With the currently accepted value of $50 \text{ km s}^{-1} \text{ Mpc}^{-1}$ for the Hubble constant, this corresponds to a distance of about 300 megaparsecs, and the angular size of the galaxy is roughly what we might expect at that distance.

An earlier attempt to find a redshift for a BL Lac object by looking for galaxy features in an extended halo had been described by Oke and Gunn (*Astrophys. J. Lett.*, **189**, L5; 1974). They used a rather different technique, employing a diaphragm to block out the light from the central object so that the galaxy lines would stand out more clearly, and their observations was of the prototype object itself, BL Lac. The absorption features they found were also very weak, but could correspond to a redshift of about 0.07.

There is however considerable doubt about the redshift in this case. More recent observations by Baldwin, Bur-

bidge, Robinson and Wampler (*Astrophys. J. Lett.*, **195**, L55; 1975) show no absorption features, and so no evidence that the nebulosity around BL Lac is made up of a normal galaxy of stars. They suggest that perhaps one reason for the difference in the results obtained is that a nearby very faint star may have contaminated the spectrum from which Oke and Gunn determined their value. Though the true nature of BL Lac must await further observational work this disagreement between two groups does highlight some of the difficulties involved.

Another BL Lac object, PKS 0735+178, is believed to have an even higher redshift and to be more QSO-like in character. A group of astronomers working in Arizona reported in June last year (Carswell, Strittmatter, Williams, Kinman and Serkowski, *Astrophys. J. Lett.*, **190**, L101; 1974) that a strong pair of absorption lines corresponding to MgII with a redshift of 0.424 had been found in this object. A number of QSOs show sharp absorption lines in their spectrum, always at redshifts lower than, or comparable to, the emission line redshift, and it is believed that in PKS 0735+178 we are seeing the same type of thing. This does not tell us the redshift of the continuum source, however, but it is extremely unlikely that it is much less than 0.424, and probable that it is at about that value.

Apart from placing BL Lac objects clearly well outside our own Galaxy, these redshift determinations can help to test our understanding of the physics



A hundred years ago

THE *Kolnische Zeitung* of Feb. 10 gives an account of Prof. Böhm's (Dorpat) researches on revival after cases of poisoning. He succeeded in reviving cats which had been poisoned by injection of potash salts into their veins, after forty minutes' duration of a state which was in no way different from actual death, the action of the heart and respiration having completely ceased. He obtained these results by artificial respiration and simultaneous compression of the breast in the vicinity of the heart. The professor points out the importance of the latter point, which he deems as essential as the action of the lungs. In any case his researches are of high interest for the relation they bear upon the revival of poisoned persons.

from *Nature*, **11**, 334; February 25, 1875

of such sources. The light and radio emission varies in intensity extremely rapidly; so rapidly that in some cases quite large changes can occur in times of only a day or two. It is very difficult to see how, without signals propagating with speeds greater than light, something can vary on time scales shorter than the light travel time across it, and so this means that the BL Lac objects emit most of their radiation from a region less than a light day or two across. Now if these objects are a very long way away, for example at what might be called 'near quasar' distances, it becomes extremely difficult to understand how the large amounts of radiation required to give the observed brightnesses can be released in so small a volume. Now that we are in a position to say that the redshifts in some cases are quite large, we have the situation where, as Jones (*Astrophys. J. Lett.*, **191**, L15; 1974) and others have pointed out, it seems likely that our understanding of the radiation processes is inadequate, or that perhaps these objects are really closer to us than their redshifts indicate.

Textured superfluids

from P. V. E. McClintock

In a recent communication from the Low Temperature Laboratory of the Helsinki University of Technology, Ahonen, Haikala, Krusius and Lounasmaa describe their transverse nuclear magnetic resonance experiments on superfluid ^3He over a wide range of pressures and temperatures down to 0.7 mK (*Phys. Rev. Lett.*, **33**, 1595; 1974). Their data seem to be consistent with the suggestion by Brinkman, Smith, Osheroff and Blount (*Phys. Rev. Lett.*, **33**, 624; 1974) that the B phase of the superfluid is anisotropic and may be expected to exhibit textures somewhat analogous to those of liquid crystals.

More than 80 papers, mostly theoretical in nature, have been written on superfluid ^3He since the original experiment in 1972 at Cornell University by Osheroff, Gully, Richardson and Lee (*Phys. Rev. Lett.*, **29**, 920; 1972) which led to its discovery. This continuing flurry of activity arises from the entirely novel states of matter which are involved.

It is now known that, below a transition temperature T_c , there are two distinct superfluid phases, referred to as A and B. The phase diagram has been mapped out by various workers and is apparently as shown in Fig. 1. Although, as can be seen, the A phase is stable only within a relatively small area of the P - T diagram, much more is now known about it than about the

lower temperature B phase. The reasons are mainly cryogenic in origin. The two previously existing methods of cooling liquid ^3He —compressional cooling along the solidification line, and adiabatic demagnetisation of the electronic spins of

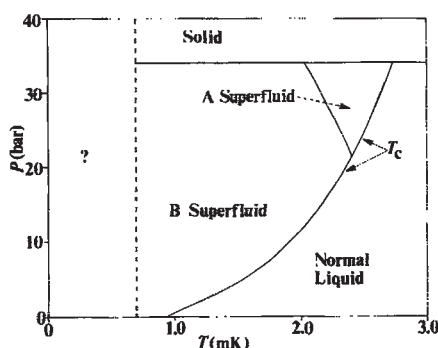


Fig. 1 The phase diagram of liquid ^3He in small magnetic fields at very low temperatures (after Paulson, Kojima, and Wheatley, *Phys. Rev. Lett.*, **32**, 1098; 1974; and Ahonen *et al.*, *Phys. Rev. Lett.*, **33**, 628; 1974). The dashed line indicates the lowest temperature to which the liquid has yet been cooled. Note that the vapour pressure at these temperatures is so small that the liquid-vapour equilibrium line comes within the thickness of the abscissa as drawn here.

cerium magnesium nitrate (CMN)—allow the whole of the A phase to be investigated, but are capable of achieving temperatures only just within the B phase. The technique of nuclear demagnetisation has been applied successfully to cooling liquid ^3He only recently, when the same group at Helsinki reported (*Phys. Rev. Lett.*, **33**, 628; 1974) that they had managed to reach 0.7 mK using this method, thus opening up the temperature range required for a detailed investigation of the B phase of the superfluid.

Why and how does ^3He become superfluid at these low temperatures? It is now almost universally believed, in spite of one or two nagging discrepancies between theory and experiment, that both A and B phases have much in common with the electron gas in a superconducting metal. In each case, the total energy of the particles, whether electrons or ^3He atoms, is reduced if they form so-called Cooper pairs. There is however an important difference in that, for superfluid ^3He , pairs are formed whose relative angular momentum is non-zero, whereas the Cooper pairs of all known superconductors have zero angular momentum. Thus, a Cooper pair in ^3He consists of two atoms orbiting around each other. Much of the experimental and theoretical effort has so far been devoted to investigating how this orbital angular momentum is correlated with the directions of the nuclear spins of the two atoms, and most of the information has been derived from experimental investi-

gations of nuclear magnetic resonance (NMR).

When a single ^3He atom is placed in a magnetic field its nucleus, which has a magnetic dipole moment of its own, precesses about the field direction in one of two permitted states, corresponding to two different energy levels. In NMR the resonant absorption of electromagnetic energy at the so-called Larmor precession frequency enables the splitting of the energy levels to be measured. When Cooper pairs are formed, however, the nuclei are no longer acting independently of each other. A careful investigation of the NMR resonant frequency, linewidth and integrated intensity, all of which are found to change from their values in normal, that is non-superfluid, ^3He therefore yields information about the way in which the nuclear spins and orbital angular momenta are correlated with each other and with possible axes of anisotropy in the liquid.

It seems to have become well accepted that the A phase is in the particular state originally proposed by Anderson and Morel (*Phys. Rev.*, **123**, 1911; 1961) with the nuclear spins of each Cooper pair lined up parallel to each other in the plane of their mutual orbit which has an angular momentum of magnitude $1\hbar$, and with the orbital momenta of all the pairs aligned and perpendicular to an externally applied magnetic field. This implies, of course, that the liquid itself will be anisotropic, that is, many of its properties will depend on the direction relative to the axis of anisotropy in which measurements are made. The Helsinki group's transverse NMR measurements are the first to be made at pressures other than on the solidification line, and it is reassuring to note that their results are in good agreement with predictions based on the assumption of the Anderson-Morel state. In particular, it seems that the pressure dependence of the shift in resonant frequency from its value in normal ^3He is well accounted for by the relation which had been derived theoretically by Leggett (*Phys. Rev. Lett.*, **29**, 1227; 1972) on the basis of that assumption.

It is the lower temperature results of the Helsinki NMR experiments, those in the relatively unknown B phase, therefore, which are in many ways the more interesting. The B phase has been tentatively identified as the state originally proposed by Ballian and Werthamer (*Phys. Rev.* **131**, 1553; 1963), in which the pair orbital angular momentum is again $1\hbar$, but for which the correlation of orbit and nuclear spins is considerably more complicated than in the Anderson-Morel state. Brinkman *et al.* have been able to demonstrate, however, that it should be possible to define a single axis of

Registry of abnormal karyotypes

AN international registry of abnormal chromosomes has been set up by the Division of Medical Genetics of Johns Hopkins University. Dr D. S. Borgaonkar and Mr D. R. Rolling have been gathering data into a computer for the past eleven months and hope to continue to receive details of new and existing human chromosome abnormalities. They aim to establish a sufficiently comprehensive collection to provide information about the prevalence of human conditions, such as Down's syndrome, which are characterised by chromosome abnormality, and to give cytogeneticists a means of exchanging data which might otherwise be lost in their files.

The computer records the name of the person reporting the karyotype, the laboratory where the work was done, the date it was reported, and the arm, region and band of the chromosome on which the rearrangement responsible for the abnormality was found. The registry is being organised on a basis similar to that of Dr Borgaonkar's book *Chromosomal Variation in Man: A Catalog of Chromosomal Variants and Anomalies* (Johns Hopkins University Press, Baltimore, in the press). The book, which was compiled with help from the Institute of Medical Research at

Camden, New Jersey, where there is a repository of abnormal karyotype cultures, and Dr F. Ruddle's laboratory at Yale University, consists chiefly of published information. The registry, on the other hand, is being compiled mostly from unpublished data. Print-outs are planned to appear two or three times a year and will be available at cost.

Dr Borgaonkar hopes that, using data in a similar way, it will be possible to catalogue 'break-points' on the mammalian X chromosome of different species to reveal 'hot spots', where unusually large numbers of breaks are reported. He also foresees catalogues of chromosome variation for species such as the common mouse, *Mus musculus*, which has well defined chromosome regions.

The response to requests for data so far has been encouraging. Some regional centres have been set up already, for example in Greece, Belgium, Moscow and Madison, Wisconsin, where reports of new abnormal karyotypes are collated and sent on to Dr Borgaonkar and Mr Rolling.

Their address is Division of Medical Genetics, Department of Medicine, John Hopkins University School of Medicine, Baltimore, Maryland 21205.

anisotropy, and that this will tend to lie parallel to an applied magnetic field.

The directions of the axes of anisotropy in both A and B phases are expected to be strongly influenced by the container walls. The reason for this is particularly easy to appreciate in the case of the A phase, for it is intuitively obvious that, unless the orbital plane of a Cooper pair is parallel to the wall, there is a danger of one of the atoms bumping into the wall and so breaking the pair. Pair-breaking requires energy, and so the liquid reduces its total energy if it orients its pairs to rotate about axes perpendicular to the walls. Although the B phase is more complicated, here, too, depairing effects determine that the axis of anisotropy must be perpendicular to the wall. In fact, of course, it is not possible, even by bending the axis, to arrange that it should meet every wall of a given container at right angles unless there exist one or more singularities at which the local anisotropy axis is undefined, and these will presumably position themselves in such a way as to minimise the total energy of the liquid, contributing to a texture which is dependent on the shape of the container.

When a magnetic field acts upon the

liquid in a container of finite size, the texture must be even more complicated. One may, however, assume that the field, provided it is strong enough, will overcome the effect of the walls in determining the direction of the anisotropy axis at positions sufficiently far from the walls: there will be a characteristic distance r_0 , dependent on field and temperature, within which the anisotropy direction moves from a wall-dominated to a field-dominated situation. Brinkman *et al.* calculated r_0 for the B phase on the assumption of a Ballian-Werthamer state and found that for $T \ll T_c$ it should be about $10/B_0$ mm with the magnetic field B_0 in mT or, in other words, comparable with the typical dimensions of a pickup coil for low field NMR. This implies that over a significant region of the sample the anisotropy axis will not be exactly parallel to the magnetic field, which will have the consequence of locally raising the frequency at which resonance occurs. The net effect is therefore an asymmetric broadening of the NMR resonance line towards higher frequencies, a broadening which should have a strong negative dependence on the magnitude of the applied magnetic field.

A field-dependent broadening of the

resonance is precisely what the Helsinki group has observed in the B phase, and although they have apparently not been able to check the detailed lineshape with that expected, the magnitude of the broadening seems to be in reasonable quantitative agreement with prediction. It was noted however, that the integrated NMR absorption was significantly lower than the calculated value. NMR measurements were also made on ^3He in the interstices of fine platinum powder, and it was observed that for small fields the signal broadened and disappeared rapidly as T was reduced below the transition temperature. This again is consistent with the picture of Brinkman *et al.*: the liquid in each void will have its anisotropy axis determined almost entirely by wall effects because the void dimensions are less than r_0 , so that the liquid takes up a domain-like structure with each domain in a different orientation relative to the applied magnetic field. The resonant frequency for each domain is therefore different so that, for the system as a whole, no resonance is seen.

Taking account of the discrepant behaviour of the integrated NMR absorption, and also in view of some earlier measurements of the static magnetic susceptibility, some doubt must inevitably remain as to whether $^3\text{He-B}$ is really in the Ballian-Werthamer state. Further experiments, in particular measurement of the specific heat for $T \ll T_c$, will be required before the question can finally be settled. There seems little doubt, however, that both of the new liquid phases of ^3He enjoy the curious distinction of being anisotropic, magnetic superfluids which display textures when contained in finite vessels.

Earth wobble, day length and continental drift

from David W. Hughes

Two well known facts have recently been combined in a rather fascinating way. The first is the period of Chandler's wobble, the second the secular decrease in the Earth's rotation period. The combination has been carried out by Cannon of York University, Toronto, Canada in a recent edition of *Physics of the Earth and Planetary Interiors* (9, 83; 1974).

The Earth is usually thought of as rotating once a day about an axis which passes through the North and South Poles. The picture is not quite so simple because the Earth is not a perfect globe but an imperfect oblate spheroid with unequal principal moments of inertia. The axis of symmetry of this oblate spheroid possesses

a degree of freedom known as wobble whereby this axis rotates about the space fixed axis of rotation. Now the wobble is very small, the Earth's pole moving around an imperfect circle of diameter about 14 m, equivalent to a 5 s arc axis movement. It can, however, be measured easily by observing carefully the passage of stars across the zenith at an observatory. This gives the colatitude of the observatory to about 0.01 s arc and the pole of the Earth can then be located to 0.3 m.

The Earth responds to the wobble excitation like a damped harmonic oscillator. Spectral analysis of the wobble data shows that part of the variation has a period of exactly one year—driven by the annual forces resulting from the seasonal redistribution of atmospheric mass, water vapour, snow and ice. The remainder of the data has periods ranging from 13 to 15 months, peaking at 428 d—this being known as the Chandler resonance. The length of the year (that is, the frequency of the annual driving force) has remained constant over geological time, whereas the Chandler resonance frequency has monotonically decreased due to the continual loss of terrestrial rotational energy by tidal interaction with the Moon.

Because of the effects of tidal friction on the dynamics of the Earth-Moon system, angular momentum from the rotation of the Earth is transferred to the orbital motion of the Moon. The day is lengthening by about 2.3×10^{-3} seconds each century and consequently the Moon moves further away, the semi-major axis of its orbit increasing by about 4 cm per year. So some time in the past the annual driving force passed through a resonance with the Chandler frequency, resulting in a large-amplitude wobble and during this resonance period geophysically significant amounts of solar energy (the Sun providing the motive power for the annual wobble) were dissipated in the upper mantle.

Resonant conditions would last throughout the time taken for the secular decrease of the Earth's rotation rate to scan the Chandler spectral band. This time interval depends on the rate of decrease of the Earth's angular velocity and on the specific dissipation of the oscillation energy in the Earth's crust. Cannon finds that resonance occurred some time between 242 and 129 million years ago, the mean estimate being 185 Myr ago. It lasted for around 5 to 25 million years. The total energy dissipated during this resonance comes to about 10^{33} erg; of the order of 10^{26} erg yr⁻¹. Considering the variation of specific dissipation with depth Cannon concludes that during this resonance most of the wobble energy was dissipated in the region of

the low velocity zone in the upper mantle, leading to a temperature increase of between 1 K and 10 K. As viscosity is dependent on the exponential of the temperature a 1 K rise causes a 5% decrease in viscosity, a 10 K rise decreasing the viscosity by 50%.

Cannon then goes on to speculate that the Chandler wobble resonance triggered continental drift, the decrease in viscosity enabling the continents to slide on the low velocity zone. Radiometric geological dating has shown that the greater part of the super-continent remained intact for as long as 2,000 Myr before the onset of continental drift. The epoch of the drift onset is thought to be 180–200 Myr ago agreeing with the time of resonance. Also the Chandler annual resonance is energetically sufficient.

Drift in interference filters

from John Walker

INTERFERENCE filters are used a great deal in optical and spectroscopic instrumentation to isolate narrow regions of the spectrum. That is, they transmit a few selected wavelengths of light, and reflect the rest. One of their drawbacks is that the passband (the region of transmission) tends to shift in wavelength as the filter ages, which can be awkward in some applications. For example, a filter with a passband 0.6 nm wide, centred at 656.3 nm, was required for the H alpha telescope in the Skylab satellite (to study hydrogen in space by means of the H alpha spectral line). Obviously, even a small shift in such a filter would make it useless.

The programme of research which produced the stable filters used in Skylab is reported in a recent publication (Title *et al.*, *Applied Optics*, 13, 2675; 1974). These workers developed a dye laser and monochromator test system which could measure the transmission of the filters and detect the size and position of any defects. Although previous studies had indicated that heat treatment and humidity could cause drift they found that ten weeks in a 100% humidity atmosphere had no effect on the filters. (One suspects that a longer test period might have caused some changes.) Heat was however found to cause drift.

To stabilise filters the manufacturer bakes them for a few hours at 100 °C. Subsequent storage at temperatures below 38 °C resulted in an average drift of the passband of 0.05 nm per year, but this increased to 2.5 nm above 80 °C, according to Title *et al.* A later set of filters, baked for much longer periods, showed no drift at all at 38 °C, a result in line with earlier work. They

were therefore used in Skylab.

An unexpected observation was that illumination also affected the filters' characteristics, and in a second paper (*Applied Optics*, 13, 2680; 1974) Title studied the phenomenon in more detail. A filter which had been exposed to solar radiation showed a drift of the passband towards shorter wavelengths. The transmission profile (as a function of wavelength) also changed, from approximately rectangular to triangular. Tests indicated that solar radiation at about 390 nm was responsible, and when a prefilter was used to cut it out the drift was eliminated. Wavelengths below 340 nm and above 500 nm did not cause any drift. Further experiments showed that longer baking times, which had stopped the 'ageing' drift, made no difference to the illumination effects.

An interference filter consists of alternate thin films of dielectrics of low and high refractive index, in this case cryolite and zinc sulphide. It is in fact a type of Fabry-Perot interferometer. Since zinc sulphide forms the interferometer cavity, and since it absorbs light at wavelengths below 500 nm whereas cryolite does not, the probability was that it rather than the cryolite was being affected by the illumination. This was confirmed by computer calculations, which showed that a decrease in the optical thickness of the zinc sulphide would not only shift the passband in the required direction, but would also result in a triangular profile, as observed.

Title did not determine whether the decrease in optical thickness was caused by a decrease in physical thickness or by a change in absorption coefficient of the zinc sulphide. Earlier work (Schneider and Räuber, *Solid State Commun.*, 5, 779; 1967; and Leutwein, Räuber and Schneider, *Solid State Commun.*, 5, 783; 1967) suggests the latter. These workers studied the optical properties and electron spin resonance of zinc sulphide crystals. Specimens which had been annealed in molten zinc showed a purple colouration, due to absorption bands at 430 and 545 nm. The colouration was enhanced by ultraviolet illumination at 355 nm, with an accompanying reduction in an absorption band at that wavelength. This is evidently the process occurring in the interference filters. Leutwein *et al.* also observed that the effect was reversible. The 355 nm absorption band was restored, and the other two eliminated, by illumination at 436 nm or by heating to 300 °C in the dark. The changes were accompanied by photoconductivity. Obviously there are two point defects in the zinc sulphide which are exchanging electrons through the conduction band. One of them, responsible for the 430 and

545 nm bands, seems to be the sulphur vacancy. The other, which causes the 355 nm absorption, was not identified. The presence of halogen or aluminium impurities also affected the spectra observed.

It therefore seems that illumination-drifted filters might be restored to their original condition by suitable illumination and/or heat treatment. Furthermore, it may be possible, by controlling the impurity content of the zinc sulphide, to make interference filters whose passband does not drift under illumination.

Crack theory developed

from Peter J. Smith

THE dilatancy model for earthquake precursors is one which in general terms attracts wide support. But as O'Connell and Budiansky (*J. Geophys. Res.*, **79**, 5412; 1974) point out, there are disagreements over detail which reveal that understanding is far from complete. For example, Nur (*Bull. seismol. Soc. Am.*, **62**, 1217; 1972) proposed that the precursory decrease in P wave-S wave velocity ratio (V_P/V_S) is due to the opening of new dry cracks in the focal zone of an earthquake, whereas Whitcomb *et al.* (*Science*, **180**, 632; 1973) noted that vaporising the fluid in saturated cracks would have the same effect. Perhaps it would be wrong to describe this as a disagreement, since it is doubtful whether either party would man the barricades to support their respective viewpoints; suffice it to say that here we have two possible mechanisms for the same phenomenon and that it is necessary to determine which, if either (and if not both), actually obtains.

What does seem to be agreed by most people is that the elastic responses of rocks in an earthquake zone are critically dependent upon the presence of internal cracks and pores and whether they are wet or dry. Yet even here the dilatancy models currently available may be incorrect in detail. As O'Connell and Budiansky again point out, current theoretical analyses of the effect of cracks on the elastic properties of solids apply only to systems in which the cracks are assumed to be so far apart that the effect of each crack on the properties of the uncracked material may be determined independently. This was the basis upon which Walsh (*J. geophys. Res.*, **74**, 4333; 1969), for example, assessed the influence of both dry and saturated cracks; and his analysis was derived, in turn, from the earlier work of Wu (*Int. J. Solids Struct.*, **2**, 1; 1966) and Eshelby (*Proc. R. Soc.*, **A241**, 376; 1957). Actual crack densities in rocks

have seldom been measured or estimated. So are dilatancy models involving low crack densities really valid?

To find out, O'Connell and Budiansky have carried out a new theoretical analysis which puts no initial restriction on the crack density and thus takes into account interactions between cracks. This freedom turns out to be critical. The model itself consists of a solid containing very thin randomly oriented ellipsoidal cracks which may be wholly dry, wholly saturated or a mixture of both. When the elastic properties predicted from the model are compared with the experimental data obtained by Nur and Simmons (*Earth planet. Sci. Lett.*, **7**, 183; 1969), who measured wave velocities in both dry and saturated rock samples at various pressures up to 3 kbar, it becomes quite clear that consistency can only be achieved by relatively high crack densities. For example, at zero pressure dry Westerly granite has a crack density of 0.25, which corresponds to one crack of diameter 1.2 units per unit volume (for example, one crack with a diameter of 1.2 mm mm⁻³). The corresponding figures for other dry rocks measured by Nur and Simmons range from 0.15 to 0.6, while in its wet state the Casco granite has a crack density as high as 0.7 (dry 0.4). Thus, all the rocks are extensively cracked and hence not strictly amenable to any theoretical treatment which presumes dilute crack concentrations.

A similar comparison is possible between the characteristics of the O'Connell-Budiansky model and the premonitory behaviour of the rocks in the region of the San Fernando earthquake of 1971—with equally interesting results. As Whitcomb *et al.* have reported, the seismic velocity ratio V_P/V_S decreased from its normal value

some 3–4 years before the San Fernando event and then gradually increased to its initial value just before the shock occurred. The original discovery of this effect was based on small earthquakes which preceded the main San Fernando shock in what was later seen to be the aftershock area and which were recorded at two stations on the same side of the aftershock zone. Subsequent work has shown that a similar result is obtained for small earthquakes occurring well outside the limits of the aftershock area but measured at two stations which have the aftershock zone between them. In other words, precursory changes in V_P/V_S are observed irrespective of whether the waves used to measure V_P and V_S pass through the main shock's epicentral zone or not.

In terms of the O'Connell-Budiansky model, however, the gross similarity in V_P/V_S behaviour does not entirely extend to the physical mechanism behind the variations. Outside the San Fernando epicentral region (original data recorded at stations on the same side of the region), the crack density before the V_P/V_S decrease lay in the range 0.2–0.3 and the cracks were predominantly saturated. Then during the V_P/V_S decrease in 1967 the crack density decreased marginally and the fluid in the cracks vaporised, which is consistent with the interpretation made originally by Whitcomb *et al.* But during the subsequent increase in V_P/V_S the crack density decreased much more noticeably to 0.15 and the cracks resaturated. This reduction in crack density is inconsistent with increased dilatancy upon resaturation, and implies instead a relaxation of strain which allows some cracks to close completely and the rest to relax sufficiently to eliminate the vapour

Getting closer to prediction

from Peter J. Smith

THE United States Geological Survey claims that "significant progress" was made towards earthquake prediction when scientists at the National Center for Earthquake Research in California managed to anticipate a moderate shock which took place on November 28 last year. This event, which had a magnitude of 5.2, occurred between the San Andreas and Calaveras faults about 16 km north of Hollister, California.

Prediction was based largely on precursory deformation of the Earth's crust and changes in the magnetic field. Crustal tilting was first observed about 4 weeks before the earthquake at two locations near what was to be the epicentre. But a "dramatic anomaly"

in the geomagnetic field in the epicentral region was spotted about 6 weeks ahead. Later analysis of recorded seismic data showed that premonitory variations in seismic wave velocity had also occurred.

According to the survey's director, Dr V. E. McKelvey, this is the first time that such a variety of precursory phenomena has been observed for a single earthquake in the United States. He warned, however, that this success should not be taken to imply that routine prediction for public safety planning is now possible. Significantly, he also pointed out that much still needs to be learned about how successful prediction could be used most effectively in reducing hazards.

phase within them. Inside the epicentral region (later data recorded at stations on opposite sides of the region), the pre-1967 crack density was slightly higher than outside but again the cracks were mostly saturated. And again the V_P/V_S decrease was accompanied by a decrease, albeit rather more marked, in crack density. But thereafter the picture changed. The subsequent increase in V_P/V_S was again related to resaturation but was now accompanied by an increase in crack density.

In summary, then, there are significant differences in the precursory processes inside and outside the immediate epicentral zone. Close to the main shock area the sequence is dilatancy-resaturation-dilatancy, whereas further away the sequence becomes dilatancy-resaturation-relaxation.

Extensive cracking is common to both regions, however, and extends over a much wider zone than some workers have previously supposed. And again contrary to some previous views, the observed decrease in V_P/V_S is not due to the formation of new cracks but to a change from saturated to dry cracks. Indeed, changes in the saturation state of the cracks are apparently more important on the whole than changes in crack numbers.

Opening up the Universe

from P. C. W. Davies

MOST cosmologists now accept that the Universe began with a bang. But how will it end? The question has been of long-standing interest to both theologians and scientists, though the former have enjoyed greater success in producing answers. In recent months, however, evidence has been accumulating from diverse astronomical sources which consistently points toward a definite scenario for the future of the Universe. Although there is no question of a unanimous verdict at this stage, a movement of opinion among the pundits is becoming perceptible.

All discussion of this matter takes place within the context of the standard model for the Universe. In this standard model, the Universe moves in compliance with Einstein's general theory of relativity. This motion is visible to us as a general pattern of expansion, and because of the apparent large-scale homogeneity and isotropy, this expansion is assumed to be everywhere uniform.

If Einstein's equations are solved for such a uniform model universe they yield a two-parameter family of motions. All of these solutions predict that the expansion began a few billion years ago, when the Universe was in a

very dense condition. The onset of the expansion, expected to be very hot, is the big bang. The past history of the Universe is therefore, in broad outline, fairly unambiguous according to this theory.

As regards the future motion of the cosmos, all the solutions predict a gradual decrease in the expansion rate. Where they differ is in whether the decrease is strong enough to arrest the expansion and bring about recontraction, with the Universe falling back on itself to end up in a bang much like the one from which it originated. The alternative is for the expansion to continue for ever, with the Universe slowly sinking into thermodynamic equilibrium, after which little of significance will occur.

In principle it is easy to decide between these alternatives. Observations of the rate of recession of distant galaxies, seen as they were in the remote past, should indicate how the expansion rate has slowed since then. Alternatively, measurements of the present energy density in the Universe enables the gravitating effect to be calculated (through the general theory of relativity) as to how vigorously this gravitation is slowing the Universe down.

In practice, both types of observation are difficult to perform and complicated by many contentious side-issues. Now a paper has appeared in the *Astrophysical Journal* (194, 543; 1975) by Gott and Gunn from Caltech and Schramm and Tinsley from the University of Texas in which many of the observations and their theoretical ramifications are examined in detail. Gott *et al.* opt for an ever-expanding (or open) universe. Some of the arguments they use were presented by Gunn at the Seventh Texas Symposium on Relativistic Astrophysics (see the report from John Faulkner, *Nature*, 253, 231; 1975).

The authors parameterise their models in terms of the Hubble parameter H_0 (expansion rate at present epoch) and Ω , the ratio of the observed density of energy to the critical density required to collapse the Universe. For an ever expanding Universe, $\Omega \leq 1$. Their figure 1 shows the constraints obtained in their paper on these parameters.

Although H_0 may be measured directly ($30 < H_0 < 120 \text{ km s}^{-1} \text{ Mpc}^{-1}$) the age of the Universe, t_0 (8 to 18 billion years), is a more severe constraint. A direct estimation of the deceleration is complicated by evolutionary effects in both galaxies and QSOs, which tend to result in an overestimation of the deceleration parameter, q_0 ($=\Omega/2$). To play safe an upper limit of 2 is placed on this parameter.

In contrast, the density (Ω) measurements tend to be underestimates, first because we only see the luminous matter in the Universe (stars, gas) and second because energy density may reside in the form of very low energy radiation (gravitons, neutrinos). Various methods for estimating Ω are critically described in the paper. Three independent estimates of the relative density of galaxies alone (denoted Ω^*) are used to obtain a value 0.05 ± 0.01 , and various arguments reviewed as to why any intergalactic matter would not be sufficient to give $\Omega > 1$.

In addition, a mention is made of recent theoretical work on the production of deuterium by nucleosynthesis in the hot big bang. The fraction of deuterium produced (D/H or ratio of deuterium to hydrogen) turns out to be very sensitive to the present energy density of the Universe. Using the results of measurements of galactic deuterium abundance, it is concluded that remarkably narrow ranges of Ω and H_0 are permitted in which $0.05 < \Omega < 0.09$ and $49 < H_0 < 65 \text{ km s}^{-1} \text{ Mpc}^{-1}$, predicting an ever-expanding Universe by a wide margin. The possibility of galactic deuterium production and nonstandard big bang physics is briefly reviewed.

The authors conclude that the density of the Universe is low, $\Omega \sim 0.06 \pm 0.02$, and a recontraction is ruled out. The most persuasive part of their argument is the fact that an ever-expanding Universe follows consistently from all the different sources of data, whereas to produce a recontracting model, a number of *ad hoc* assumptions are necessary. It is always possible to invoke exotic processes in the big bang, or to conjecture that most of the mass of the Universe is in the form of undetectable gravitational waves or black holes. But such conjectures are extremely hard to falsify with current technology, and seem somewhat contrived.

If Gott *et al.* are right, then instead of the Universe going out in a blaze of glory by recontraction, collapse and final cremation, it is doomed to everlasting frozen stagnation, when the stars go out in a few dozen billion years.

Erratum

IN the article "Chilling Statistics on Cyprus" by Peter J. Smith (*Nature*, 253, 500; 1975) magnetic vectors are mentioned in the last sentence of the fourth paragraph. This is incorrect and the sentence should read: "In other words, on each side of the intrusion zone the chilled margins will all lie in the same direction (they will all be 'one way') and the degree of 'one way chilling' will be 100%."

articles

Reconnaissance Rb/Sr isochron study in the Bergen Arc System and regional implications

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On the basis of reconnaissance geochronological investigations, and the preliminary results of the reinvestigation of the tectono/metamorphic patterns of the Bergen Arc System it is possible to present a series of results which help to clarify the relationships of the rocks units involved in this system. These results fundamentally affect the interpretation of the Bergen Arc System in Caledonian reconstructions of the geology of Western Norway.

THE rocks of the Bergen Arc System consist of a series of arcuate belts both of Lower Palaeozoic metasediments and metavolcanics and of gneisses including migmatites and the anorthositic rocks of the Bergen-Jotun kindred¹⁻³ (Fig. 1). The age relationships of the various members of the arc system have been a matter of debate and various models have been proposed to explain them. Kolderup and Kolderup³ concluded that all the arc rocks east of the Øygardens gneiss area were of Caledonian development, a thesis supported by Hernes⁴ as recently as 1967. He further concluded that the Bergen Arcs represent a continuous stratigraphical sequence from Precambrian gneisses through late Precambrian metasediments and migmatites (The Minor Bergen Arc and Ulrikens Gneiss), late Precambrian metavolcanics (the anorthosites and associated rocks) into fossiliferous Lower Palaeozoic rocks (the Major Bergen Arc). Hernes supports this proposal by stating that the arc sequence can be correlated with a corresponding sequence in the Møre area. But Kvale⁵, following Reusch¹ and Werenskiöld², was convinced that the anorthositic and associated gneissic rocks represented an allochthonous thrust unit which overlies and separates the Major and Minor Bergen Arcs with their sequence of Lower Palaeozoic metamorphic rocks. Recent field and geochronological studies support Kvale's proposition, though the pattern of thrust tectonics is more complex than he then envisaged.

This account deals with a limited section of the arc system from the western basement gneisses of Sotra to the rocks of the anorthosite complex.

Øygarden region

The basement of the Sotra (Øygarden) region is represented by a series of granitic, granodioritic and tonalitic gneisses with amphibolites. Field studies show that these rocks have undergone several phases of reworking, under amphibolite facies conditions, that involve at least two stages of anatexis mobilisation. Several localities were sampled in this basement region and quartzo-feldspathic gneisses from the north of Sotra give an Rb/Sr isochron of $1,750 \pm 60$ Myr (Table 1), which represents the oldest age yet obtained from the basement to the Bergen

Arc system. This isochron age fits into the general pattern of the Svecofennian age previously recorded in the Baltic Shield⁶, and conforms with both the pattern of Svecofennian ages in the Nordland district⁷ and recent determinations from the basal/gneiss region in the Kristiansund area (Table 2). It also corresponds to the first Laxfordian metamorphism (LM 1) of the Scottish Lewisian⁸. In their original memoir Kolderup and Kolderup³ drew attention to gneissic rocks in

Fig. 1 Generalised geological map of the Bergen Arc System. Numbers refer to data given in Tables 1 and 4 and Fig. 2.

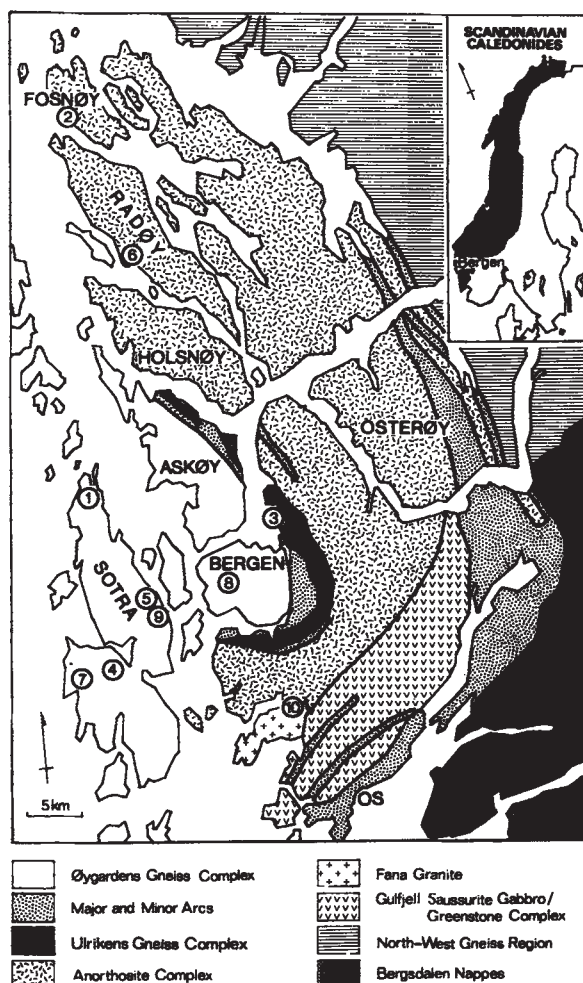


Table 1 Rb/Sr whole rock isochron ages from the Bergen Arc region

Rock type	Locality	Isochron (Myr)	Initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio (IR)	Source
1 Quartzofeldspathic gneisses	North Sotra	1,750 ± 60	0.7015 ± 0.0020	This study
2 Partially retrograded quartzofeldspathic granulite	Fosnøy	1,775	0.704	Preliminary data
3 Anatectic granite	Sandviken, Bergen	1,440 ± 100	0.7048 ± 0.0026	This study
4 Granite-gneiss	Selsta, Sotra	1,024 ± 85	0.711 ± 0.005	This study
5 Foliated quartz-syenite	Ekrhovda, Sotra	1,042 ± 92	0.718 ± 0.002	This study
6 Mangerite	Manger	1,064 ± 24	0.7030 ± 0.0017	ref. 19
7 Fine-grained granite dyke (boudin)	Telåvåg	800 ± 14	0.731 ± 0.002	This study
8 Intrusive granite veins	Bjørndalstre, Laksevåg	890 ± 150	0.730 ± 0.007	This study
9 Medium grained granite	Ekrhovda, Soltra	473 ± 31	0.754 ± 0.002	This study
10 Fana granite	Fana	453 ± 50	0.7063 ± 0.0037	ref. 15
11 Rhyolite	Kattnakken, Stord	455 ± 5	0.7071 ± 0.0018	ref. 16

the south of Sotra which have the mineralogy of quartzofeldspathic granulites; in the northern Sotra area relict granulite facies assemblages are found in several localities within boudins and show varying degrees of degradation to amphibolite facies. The low initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio for this 1,750 Myr isochron suggests that this may be an original age rather than a metamorphic age. But on the basis of one isochron all that can be said is that this represents a minimum age for the gneisses, and that the granulite facies relicts could possibly indicate an older event.

In the central part of Sotra and extending onto the mainland at Løvstakken is a later zone of amphibolite facies foliation reactivation. A series of samples were collected from this zone both of the reworked gneisses and of leucocratic dykes and veins cutting them (Table 1). The results of the Rb/Sr isochron study (Tables 1 and 4, Fig. 2) yield the following results: (1) Granitic gneisses from Selsta give an isochron age of $1,024 \pm 85$ Myr. and three biotite whole-rock pairs give respectively 598 Myr, 530 Myr, and 402 Myr. (2) A foliated quartz-syenite sheet intruding augen gneisses at Ekrhovda gives a whole-rock isochron at $1,042 \pm 92$ Myr and K-feldspar/whole rock ages of 546 Myr and 550 Myr. (3) A boudined highly radiogenic granite dyke, intrusive into these gneisses at Telåvåg yields a 'two-point' age of 800 ± 14 Myr. (4) Granite veins intruding foliated amphibolites at Bjørndalstre yield a whole-rock isochron age of 890 ± 150 Myr. (5) A boudined dyke of medium grained granite from Ekrhovda yielded a whole-rock isochron of 473 ± 31 Myr, with two biotite whole-rock ages of 398 Myr and 395 Myr.

It is concluded that this zone of structural/metamorphic reworking represents localised effects of Grenville (Sveco-Norwegian) reactivation of the older basement complex. Indeed the two isochron results at 1,024 Myr and 1,042 Myr, with relatively high initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios, conform well with the patterns obtained from Telemark^{13,14} and other west coast regions to the south which lie in the main zone of Grenville regeneration of the Southern Norwegian basement. Similar ages are seen in the nappe areas and the north west gneiss region (Table 3). The 890 and 800 Myr isochrons were determined on later minor granitic intrusions emplaced into this zone after the main Grenville reworking had ceased and again may be compared with similar intrusions from Telemark¹³. They are both now boudinaged, but this is likely to be an effect of Caledonian strains in the area. The youngest isochron age of 473 ± 31 Myr

from a boudinaged dyke is almost certainly an intrusive age and coincides closely with an isochron age of 453 ± 50 Myr obtained by Brueckner¹⁵ from the Fana granite and an isochron age of 455 ± 5 Myr for rhyolitic rocks¹⁶ in the Lower Palaeozoic volcanic complex of the island of Stord, south of Bergen (Table 1, Fig. 2). The deformation of this dyke occurred during the late Caledonian reworking of the basement complex. The Øygarden gneisses were regarded by Kolderup and Kolderup³ as being of Precambrian age, a view restated by others^{4,5}, and indeed indicated from K/Ar determinations¹⁷. The results of the present study abundantly confirm this view.

Ulriken and Manger region

The basement gneisses of the Øygarden area are structurally overlain by the rocks of the Minor Bergen Arc which mainly comprise metasedimentary and meta-igneous rocks of supposed Lower Palaeozoic age^{3,5}, although the Minor Bergen Arc also includes a number of zones of gneissic rocks which have strongly mylonitic fabrics and possibly represent imbricated slices of the underlying basement. As yet no geochronological studies have been carried out on these rocks.

Structurally above the rocks of the Minor Bergen Arc there are the migmatitic gneisses of the Ulriken's gneiss zone, separated from the former by a considerable thickness of blastomylonites. These gneisses have previously been described as Caledonian migmatites³ with associated metasediments. Recent mapping (A. Thon, and T. Sylvester, personal communication) shows that this is a highly complex zone of gneissic rocks comprising at least three thrust units of migmatitic gneisses separated by zones of younger metasediments and blastomylonites. The migmatitic gneisses show a complex history of repeated migmatization separated by deformational phases and periods of emplacement of minor basic intrusives as dykes and sheets. To make a preliminary assessment of the age of these gneisses samples were collected from a series of well-marked anatectic veins which cut foliated basic sheets. The basic sheets cut earlier migmatitic structures which contain deformed basic schlieren and quartzofeldspathic vein material, thus showing that the sampled veins represent a late stage in the tectono-metamorphic development of the gneisses. The result of the first Rb/Sr isochron study from these rocks yields an age of $1,440 \pm 100$ Myr for the late anatectic veins, and shows clearly that the gneisses of the Ulriken's gneiss zone are of Precambrian age. The age of this anatexial-metamorphic event may conform with a recent result of $1,550 \pm 100$ Myr for the overthrust gneisses in the Hardangervidda area^{18,21} and would seem to correspond to the second Laxfordian metamorphism (LM 2) in the Scottish Lewisian⁹. The possibility that the gneisses of the Ulriken's gneiss zone are considerably older than this must not be ruled out, especially as the isochron of 1,440 Myr was obtained on late anatectic veins which postdate an involved sequence of metamorphic development in the gneisses.

The gneisses of the Ulriken's gneiss zone are separated from the structurally overlying rocks of the anorthosite association by a marked development of blastomylonitic rocks and the two zones appear to represent different structural-metamorphic units, as indeed was indicated by earlier workers in the region^{1-3,5}. The results of an Rb/Sr isochron study of mangerites,

Table 2 Svecofennian-Laxfordian Rb/Sr isochron ages from other parts of Western Norway

Rock type	Locality	Isochron age (Myr)	Source
Leucocratic gneisses	Dovre fjell	1,880	ref. 15
Quartzofeldspathic gneisses	Kristiansand	1,708 ± 60	ref. 8
Quartzofeldspathic gneisses	Egersund	1,620 ± 100	ref. 24
		1,420 ± 100	
Quartzofeldspathic gneisses	Hardangervidda	1,643 ± 88	ref. 18
Augen gneisses	V. Agder	1,451 ± 53	ref. 20
Granitic gneisses	Hardangervidda	1,550 ± 100	ref. 21
Various gneisses	Sunnmøre	1,682 ± 70	ref. 33

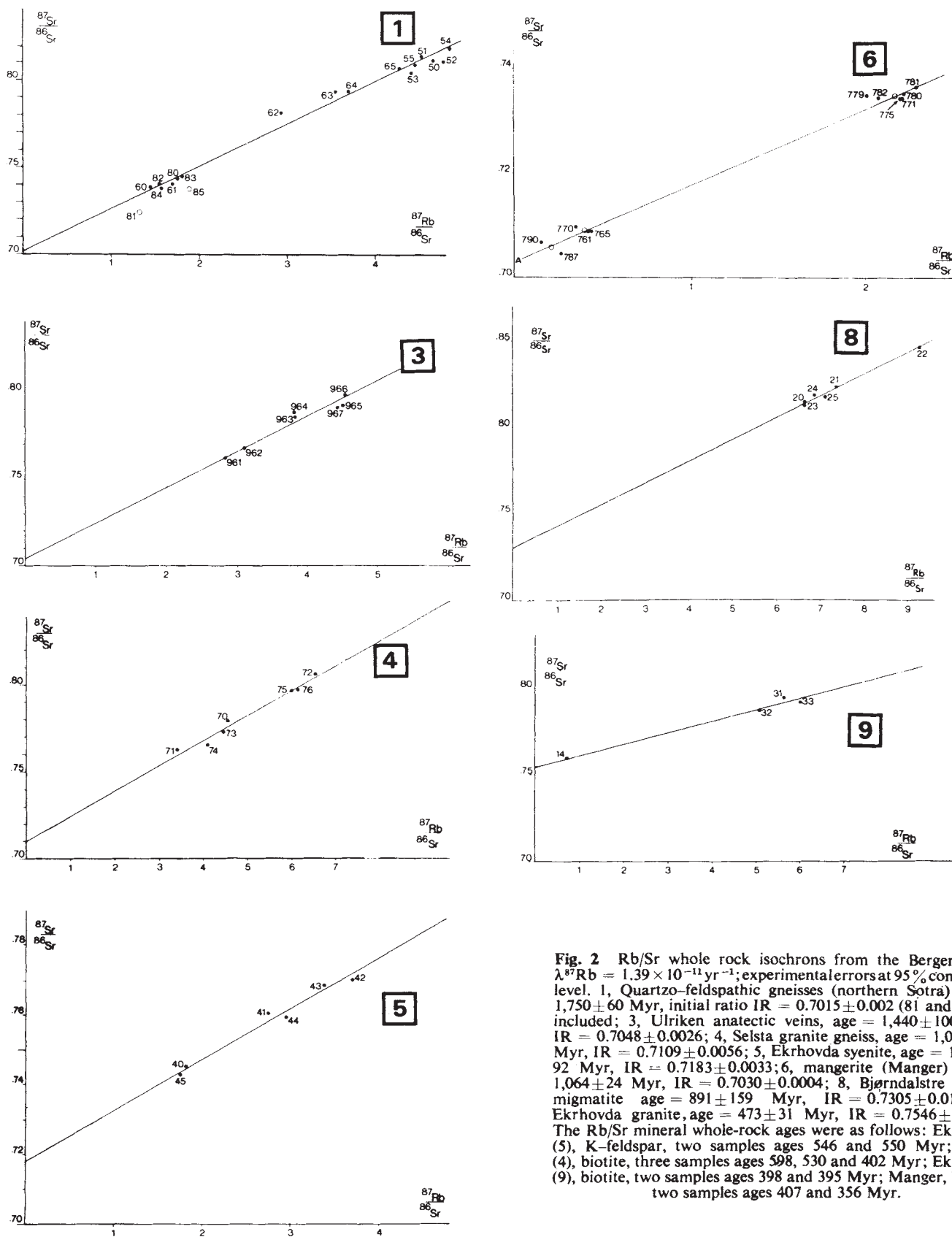


Fig. 2 Rb/Sr whole rock isochrons from the Bergen Arcs. $\lambda^{87}\text{Rb} = 1.39 \times 10^{-11} \text{ yr}^{-1}$; experimental errors at 95% confidence level. 1, Quartz-feldspathic gneisses (northern Sotra) age = $1,750 \pm 60$ Myr, initial ratio IR = 0.7015 ± 0.002 (81 and 85 not included); 3, Ulriken anatectic veins, age = $1,440 \pm 100$ Myr, IR = 0.7048 ± 0.0026 ; 4, Selsta granite gneiss, age = $1,024 \pm 85$ Myr, IR = 0.7109 ± 0.0056 ; 5, Ekrhovda syenite, age = $1,042 \pm 92$ Myr, IR = 0.7183 ± 0.0033 ; 6, mangerite (Manger) age = $1,064 \pm 24$ Myr, IR = 0.7030 ± 0.0004 ; 8, Bjørndalstre granite migmatite age = 891 ± 159 Myr, IR = 0.7305 ± 0.016 ; 9, Ekrhovda granite, age = 473 ± 31 Myr, IR = 0.7546 ± 0.0018 . The Rb/Sr mineral whole-rock ages were as follows: Ekrhovda (5), K-feldspar, two samples ages 546 and 550 Myr; Selsta (4), biotite, three samples ages 598, 530 and 402 Myr; Ekrhovda (9), biotite, two samples ages 398 and 395 Myr; Manger, biotite, two samples ages 407 and 356 Myr.

Table 3 Grenville (Sveco-Norwegian) Rb/Sr isochron ages from other parts of West Norway

Rock type	Locality	Isochron age (Myr)	Source
Granite gneisses	Tafjord	1,060±160	ref. 35
Foliated granodiorite	Hamlagrovatnet	1,004±100	ref. 15
	Bergsdalen		
Granite gneisses	Holsnevatnet (Sunnfjord)	1,253±100	ref. 15
Schist and gneiss	Almklovdalen	1,150	ref. 15
Granite gneisses	Stavanger	1,160±24	ref. 12
Gneisses of Dyrskard group	Hardangervidda	1,289±89	ref. 18
Valldalen supercrustals	Hardangervidda	943±100	ref. 18
Hestbrepiggen granite	Jotunheimen	1,033±37	ref. 13
Augen gneisses	Kristiansand	1,113±106	ref. 11
Late mangerites	Egersund	1,025±13	ref. 10
Vrådal granite	Telemark	908±49	ref. 13

from the type area at Manger, have already been published¹⁹, and show an isochron age of 1,064±24 Myr which was concluded to represent the age of granulite facies metamorphism of these rocks. This seems to show a Grenville (Sveco-Norwegian) age of metamorphism and fits well determinations made on mangerites in the Egersund region²⁰ both in terms of whole-rock Rb/Sr isochron studies and U/Pb on zircons. The rocks of the anorthosite association have been correlated with those of the Jotun Nappe (as part of the Bergen-Jotun Kindred^{3,5}). No results are available from equivalent rocks in the Jotun Nappe,

but Priem²² has dated the Hestbrepiggen Granite, in this nappe, at 1,033–37 Myr. The work of Banham²³ shows that this granite has undergone deformation and metamorphism. Two K/Ar mineral ages are available on feldspars from pyroxene gneisses at 1,280±30 and 1,020±40 from the north-western part of the Jotun Nappe²⁴. With the limited data available it is only possible to suggest the coeval nature of the metamorphism in the two situations. Two Rb/Sr biotite whole-rock determinations made on the mangerites from Manger and these gives ages of 407 and 356 Myr.

Fosnøy region

Recent mapping of the Fosnøy region in the northern part of Kolderup and Kolderup's Anorthosite association³ shows the geological situation of these rocks is much more complex than has previously been envisaged. Rocks of the anorthosite association *per se* are separated from an amphibolite facies gneiss complex which includes migmatites metasediments and metabasites, hereafter termed the 'Fosnøy gneiss complex' by clearly marked zones of blastomylonite and porphyroclastic gneiss up to 50 m thick. The structural relationships of the two rock complexes show an intricate folding of the interface including the blastomylonites, by at least two folding phases of presumed Caledonian age, the earliest of which has produced a large sub-isoclinal fold of the contact. The amphibolite facies rocks of the Fosnøy gneiss complex represent strongly downgraded granulite facies rocks. Relicts of the granulite

Table 4 Analytical data for new dates reported. Experimental uncertainties²⁰ are taken as 1.5% and 0.15% for the ⁸⁷Rb/⁸⁶Sr and ⁸⁷Sr/⁸⁶Sr ratios respectively. (⁸⁷Rb/⁸⁶Sr ratios for all minerals and whole rock samples from Fosnøy, Sandviken and Manger were determined by isotope dilution analysis, the others by X.R.F. analysis)

Sample	Rb (p.p.m.)	Sr (p.p.m.)	⁸⁷ Rb/ ⁸⁶ Sr(At)	⁸⁷ Sr/ ⁸⁶ Sr(At)	Sample	Rb (p.p.m.)	Sr (p.p.m.)	⁸⁷ Rb/ ⁸⁶ Sr(At)	⁸⁷ Sr/ ⁸⁶ Sr(At)
1 North Sotra-quartzo-feldspathic gneisses					5 Ekrhovda-foliated quartz-syenite				
50WR	175.0	106.0	4.64	0.8118	40WR	121.0	188.0	1.80	0.7451
51WR	172.0	107.0	4.50	0.8144	41WR	137.0	141.0	2.73	0.7606
52WR	178.0	105.0	4.76	0.8139	42WR	162.0	123.0	3.69	0.7702
53WR	177.0	113.0	4.39	0.8050	43WR	153.0	127.0	3.38	0.7689
54WR	176.0	103.0	4.82	0.8180	44WR	133.0	126.0	2.94	0.7595
55WR	173.0	110.0	4.43	0.8097	45WR	115.0	184.0	1.74	0.7429
60WR	142.0	276.0	1.44	0.7384	41Kspar	349.0	148.0	6.94	0.7927
61WR	165.0	274.0	1.69	0.7404	42Kspar	352.0	135.0	7.64	0.8005
62WR	165.0	159.0	2.92	0.7813	6 Manger-mangerite				
63WR	166.0	132.0	3.54	0.7945	761WR	92.12	639.7	0.41	0.7086
64WR	172.0	132.0	3.67	0.7945	765WR	92.02	628.3	0.42	0.7089
65WR	181.0	120.0	4.26	0.8077	770WR	76.01	638.1	0.34	0.7094
80WR	109.0	174.0	1.74	0.7435	771WR	188.9	246.2	2.21	0.7350
81WR	97.1	208.0	1.30	0.7244	775WR	188.9	246.2	2.21	0.7350
82WR	97.8	178.0	1.53	0.7406	779WR	180.5	258.1	2.01	0.7354
83WR	112.0	175.0	1.79	0.7455	780WR	185.4	240.7	2.22	0.7360
84WR	122.0	219.0	1.55	0.7375	781WR	206.6	259.4	2.29	0.7370
85WR	132.0	198.0	1.87	0.7385	782WR	183.8	255.1	2.07	0.7335
2 Fosnøy-quartzo-feldspathic gneisses					787WR	173.8	1941.0	0.26	0.7044
7314WR	16.8	243.1	0.20	0.7091	790WR	37.92	788.4	0.14	0.7069
7315WR	37.4	1791.0	0.06	0.7053	761Bi	396.1	43.89	26.2	0.8367
7316WR	16.9	9.571	0.05	0.7056	765Bi	340.8	46.79	21.2	0.8268
7319WR	66.8	1856.0	0.10	0.7065	A Associated Anorthosites				
3 Sandviken-anatectic granite								0.01	0.7032
960WR	172.2	130.6	3.80	0.7869	7 Telåvåg-boudinaged granite dyke				
961WR	165.4	168.7	2.82	0.7612	1WR	358.0	126.0	8.02	0.8250
962WR	160.7	149.7	3.09	0.7670	2WR	288.0	19.5	42.3	1.2084
963WR	174.9	132.2	3.81	0.7846	8 Bjørndalstre-granite migmatite				
965WR	191.8	123.4	4.48	0.7907	20WR	169.0	66.9	6.66	0.8123
966WR	196.0	125.0	4.52	0.7965	21WR	179.0	63.2	7.39	0.8229
967WR	187.8	122.2	4.43	0.7899	22WR	211.0	64.2	9.29	0.8458
4 Selsta-granite gneiss					23WR	164.0	70.1	6.66	0.8141
70WR	196.0	121.0	4.55	0.7793	24WR	159.0	65.9	6.88	0.8187
71WR	207.0	170.0	3.40	0.7623	25WR	172.0	73.1	7.15	0.8173
72WR	225.0	96.0	6.53	0.8065	9 Ekrhovda-granite				
73WR	239.0	152.0	4.41	0.7728	14WR	28.8	118.0	0.71	0.7593
74WR	262.0	181.0	4.09	0.7652	31WR	194.0	101.0	5.62	0.7945
75WR	226.0	106.0	6.00	0.7965	32WR	185.0	107.0	5.07	0.7874
76WR	228.0	105.0	6.14	0.7968	33WR	210.0	102.0	6.00	0.7916
71Bi	861.0	12.4	253.0	2.8467	31Bi	877.0	14.6	192.0	1.8295
72Bi	1134.0	16.6	233.0	2.0741	32Bi	818.0	14.1	191.0	1.8101
75Bi	911.0	12.9	255.0	2.6375					

facies assemblages are variably preserved. The first amphibolite facies foliation in these rocks has been subjected to three phases of folding before the emplacement of a set of granite aplite dykes which themselves predate the formation of the blastomylonites developed at the contact with the rocks of the anorthosite association.

To obtain an assessment of the age of the Fosnøy gneiss complex we collected samples from both the granulites and the aplite dykes mentioned above. Unfortunately both suites of samples have very low Rb/Sr ratios and the dykes in particular have a very small spread of these ratios. On the basis of preliminary results so far obtained from partially downgraded granulites, it is only possible to say that they are suggestive of an age of approximately 1,775 Myr. This approximate age corresponds with ages obtained for the older gneisses on Sotra (Table 1) and indicates them to be of Sveco-Fennian/Laxfordian (LM I) age. Whether or not this represents the age of granulite facies metamorphism or the amphibolite facies retrogression of the granulite facies rocks is not possible to say at this stage of the investigation.

Significance for the Bergen Arc System

The results of the present reconnaissance Rb/Sr isochron study of the western portion of the Bergen Arc System show a series of important results. First, the basement gneisses of the Øygarden are confirmed as being of Precambrian age, with minimum age approximately 1,750 Myr. This corresponds to the main Sveco-Fennian event of the Baltic Shield, though the presence of relicts of down-graded granulite facies assemblages in the rocks of Sotra suggest that this basement complex may be even older. The results further show that the basement of the Øygarden has been reworked during the Grenvillian (Sveco-Norwegian) event at 1,000–1,200 Myr and has subsequently been intruded by granitic dykes at around 800–900 Myr. These results demonstrate the complex sequence of deformational/metamorphic events which have occurred in the basement substrate of the Bergen Arc System before the Caledonian Orogeny. The results from the Ulriken gneiss complex, the Anorthosite association and the Fosnøy gneiss complex show that substantial parts of the thrust nappes overlying the Lower Palaeozoic schists of the Minor Bergen Arc represent overthrust masses of Precambrian basement rocks, and furthermore that they are probably derived from significantly different portions of the basement substrate to the Caledonides. The age of 473 ± 21 Myr for a late granitic dyke in the basement conforms with the age of other granitic activity, during Ordo-Silurian times, within the general region.

The mineral ages are metamorphic ages representing the effect of Caledonian reworking on the Precambrian rocks investigated. The group of mineral ages in the range of 500–590 Myr probably represents either an incompletely reset last event or incompletely reset ages related to the early Caledonian-Grampian event. The group around 400 Myr certainly seems to fit with the well documented late/end Silurian event recorded in most parts of the Caledonides; this is also the stage at which the major transport of the thrust nappes occurred. The latest age at 356 Myr may possibly reflect movements affecting the Devonian rocks to the north of the Bergen Arc region, but inference from one biotite age is only tentative.

Brueckner¹⁵, in reviewing the age patterns from Precambrian rocks in southern and western Norway, makes strong appeal to the lack of continuity of the Karelian-Laxfordian age province in this region, and suggests that there is a lack of fit between the Baltic and Greenland Shields across the continental best-fit reconstruction of Bullard *et al.* This is based on the lack of suitable age determinations in the 1,500–1,800 Myr range south of latitude 62° N in Norway. Since Brueckner's paper, however, several results within this range have been reported (Tables 1 and 2). There are several U/Pb zircon ages within this range^{8,20}, and also K/Ar ages for amphiboles and pyroxenes from the NW gneiss area^{26,27}. Thus it seems that there is considerably more continuity of the Ketilidian-Laxfordian age

province into western and south-western Norway than has previously been suggested¹⁵, though the age pattern has obviously been rendered more complex by the varying intensity of Grenvillian (Sveco-Norwegian) reworking.

On the basis of the Rb/Sr isochron ages at present available it is interesting to speculate on the possible form of the Grenville orogenic configuration in pre-Caledonian times in the North Atlantic region. Traditionally the Grenville-Sveco-Norwegian zone has been projected eastwards from Canada across Scotland into the southern part of Norway, swinging sharply into a NNW-SSE trend in south-eastern Norway and south-western Sweden^{7,28}. Evidence we present here, and other data¹⁵, shows that the effects of the Grenville-Sveco-Norwegian regeneration also affects basement gneisses in the north-west gneiss area of Western Norway (Table 3). The presence of Grenville-Sveco-Norwegian metamorphic ages in the thrust complexes of Western Norway (see also refs 12, 22), which are regarded as having been thrust from a north-westerly direction, poses the intriguing possibility that a major arm of the Grenville orogenic zone previously extended through the northern part of the North Atlantic passing between Greenland and Scandinavia. Several Rb/Sr isochron ages, in the general range 1,000–1,200 Myr have recently been established on the seaboard margins on either side of the northern North Atlantic area. The possibility of an isotopic event at approximately 1,200 Myr in northern Norway has been proposed recently for rocks from the Nasafjell window in Nordland⁷ and from the Lofoten Islands²⁹. In Western Finnmark an age of metamorphism of approximately 1,030 Myr has been indicated for gneisses within the Caledonian belt^{30,31}, and on the western side of the Caledonian orogene Rb/Sr isochrons, K/Ar mineral ages and U/Pb zircon ages in the range 1,060–1,194 Myr have recently been published^{32,33}, for westward thrust basement slices within the Caledonides of the Scoreby Sound region of East Greenland.

Though much more comprehensive geochronological work is obviously required to substantiate this proposition, it seems necessary at least to speculate on the possible presence of a northern arm of the Grenville orogenic zone extending between Greenland and Scandinavia more or less along the site of part of the subsequent Caledonian orogene. The patterns of published age determinations from the continental blocks of Greenland and Scandinavia indicate that the effects of such basement reactivation and metamorphism are restricted to the western seaboard of Scandinavia and within overthrust masses on the respective sides of the Caledonian orogene.

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Missing link between Milankovitch and climate

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A simple empirical model shows that Earth's surface temperature is most affected by seasonal change of irradiation in the interior of North America and Euroasia, and that the highest sensitivity to insolation is reached in the autumn. Past climates are explained by adjustment of the cryosphere to changes of insolation in early autumn.

It has been suggested that the gross changes in Pleistocene climate were controlled by variations in the Earth's orbit, often referred to as Milankovitch's mechanism¹⁻³. Radiometrically dated palaeoclimatic evidence strongly supports such a conclusion. The periodicity in the occurrence of gross cold and warm peaks during the past 150,000 yr matches that of the precession cycle⁴. The process affects only the seasonal and geographical distribution of the irradiation, yearly totals remaining constant. Surplus in one season is compensated by a deficit during the opposite one; surplus in one geographical area is compensated by simultaneous deficit in some other zone. If the mechanism influences climate, a sensitive area and/or season must exist in which heat budgets are modified by changes in incoming radiation to a larger extent than in the rest of the globe and/or during the rest of the year.

Climatologists of the Milankovitch school have seen such a zone in the high latitudes of the northern hemisphere where Pleistocene continental glaciers repeatedly developed and vanished. The sensitive season was considered to be summer because contemporary mountain glaciers would melt during warm dry summers but grow during the cool wet ones. Without further analysis, it was presumed that cool summers would result from lowered insolation and Milankovitch's tables³ as well as their modern counterparts^{5,6} were calculated as time series of zonal irradiation totals for summer half year (spring and summer) and winter half year (autumn and winter).

Today costly mathematical models are used to test the effects of Milankovitch's mechanism on climate^{7,8}. It seems appropriate, therefore, to check the assumption on the decisive climate forming role of summer and examine the geographical relationships of seasonally changing insolation and temperature with recently available weather data. My objective is to localise the area and season which is exceptionally sensitive to insolation change.

Basic assumptions

The ability of the atmosphere and ocean to store and redistribute energy makes an analysis of any local heat budget

rather complicated. We do not have sufficient knowledge and resources to attempt a complete study involving all the elements of the Earth's heat budget on a global scale.

But we can try to identify the sensitive areas and seasons as such, where and when the change in the local ground level irradiation is paralleled with least delay by the largest change in surface temperature.

This approach should be correct if two basic assumptions involved are correct, namely:

(1) That the energy transformation at ground level is of primary importance in determining the climatic state, while the variations in direct solar heating of the atmosphere and in the escape of long wave radiation have only contributory effects on the ground level climate⁹.

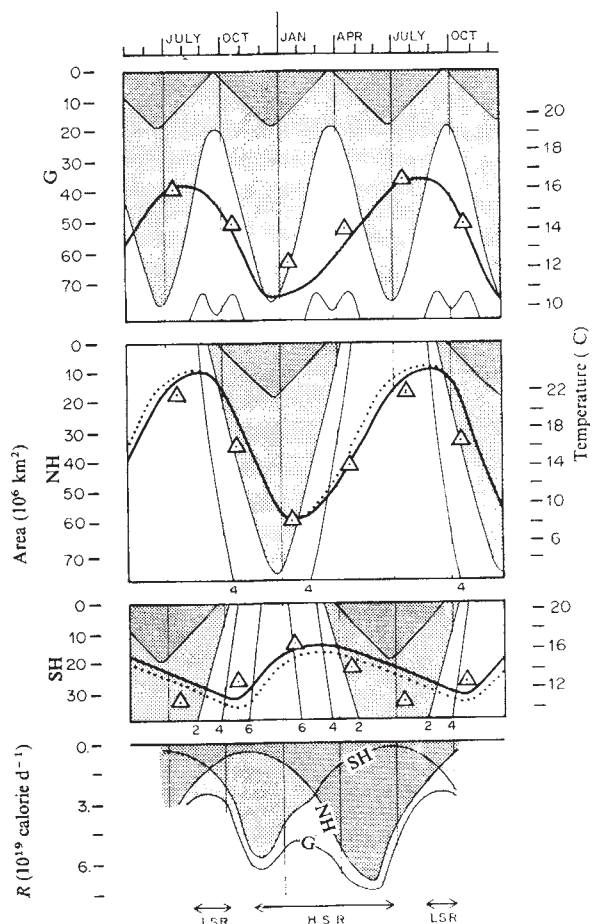


Fig. 1 Mean monthly surface air temperatures (triangles) for the globe (G), Northern Hemisphere (NH) and Southern Hemisphere (SH) compared with extent of cryosphere T_0 (dotted line), snow and ice cover (full line), and Q_0 (heavy dots), Q_{200} (light dots), Q_{400} and Q_{600} areas (4 and 6 respectively). Also shown are seasonal changes in global R (reflection from snow and ice assuming cloudless days). The seasonal wave in mean global temperatures is controlled by the Northern Hemisphere and the mean global and NH temperatures inversely correlate with the extent of cryosphere.

Table 1 Monthly means* of Q , T , and S

	Globe			Northern Hemisphere			Southern Hemisphere		
	Q langley d^{-1}	T $^{\circ}C$	S $10^6 km^2$	Q langley d^{-1}	T $^{\circ}C$	S $10^6 km^2$	Q langley d^{-1}	T $^{\circ}C$	S $10^6 km^2$
January	560	12.2	76	365	8.0	58.4	755	16.5	18
April	570	14.1	59	570	14.2	41.2	570	14.1	18
July	530	16.1	42	725	21.6	14.3	335	10.6	28
October	570	14.3	57	570	16.2	22.8	570	12.4	34
Mean	560	14.2	60	560	15.0	34.8	560	13.4	25

* Q , After Berland¹⁰; T , long term means after Shutz and Gates¹¹; satellite observed mean S areas covered for at least five consecutive days in the Northern Hemisphere during 1967-73 interval and in the Southern Hemisphere in 1968 (ref. 14)

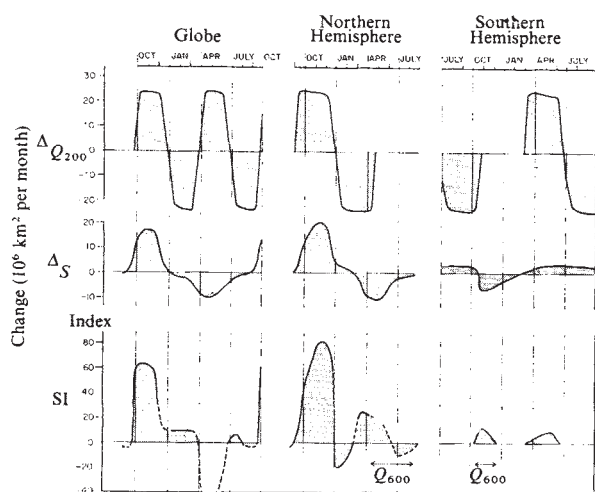


Fig. 2 Rates of increase (positive values) and decrease (negative values) of S and Q_{200} areas through the year. Sensitivity index SI shows the proportion between ΔS and ΔQ_{200} after the formula $SI = (\Delta S / \Delta Q_{200}) \times 100$. High positive index indicates high sensitivity of climate to insolation change, as observed in the Northern Hemisphere from September through November. Q_{600} marks intervals where ΔQ_{200} was substituted in the formula by ΔQ_{600} . Dotted line through areas where ΔS or ΔQ approaches zero.

(2) That the atmospheric and oceanic circulation in general tends to smooth and compensate, rather than strengthen the geographic and temporal differences in the heating of the globe. Thus, the longer the delay between the local irradiation change and climatic response, the greater the influence of compensatory processes related to lateral heat transport, and the lower the sensitivity of local heat budget to the insolation input at any moment.

The QTS-1 model

QTS-1 is a highly simplified one-level global climate model in which heat transfer in the atmosphere and oceans as well as the hydrologic cycle are completely disregarded. It is named after the main recognised variables: ground level irradiation Q , surface air temperature T , and the area of snow and ice cover S . The 'instant' climate state in the model is defined by the mean surface air temperature T . Extensive parametrisation of empirical input data was used in the present first version of the model, QTS-1. Improved variants will be studied in the future.

The sources of input data and the way they were parametrised are as follows:

Q . Daily totals of solar radiation incident upon the Earth's surface under cloudless sky, accounting for zonally averaged mean turbidity¹⁰. Units are langley d^{-1} , where 1 langley = 1 calorie cm^{-2} . Related measures are Q_0 , the area in million km^2 with no irradiation income; and Q_{200} , Q_{400} , and Q_{600} , the areas where irradiation is below 200, 400, and 600 langley d^{-1} respectively. Cloud effect on Q is omitted.

T . Surface air temperature ($^{\circ}C$) (refs 11–13). T_0 is the area with temperatures below $0^{\circ}C$, corresponding roughly to the ground level extent of the cryosphere.

S . Snow and ice covered area irrespective of snow density in million km^2 . Data from the northern hemisphere obtained from NOAA weekly maps of average snow and ice boundaries, all other data as listed in ref. 14. Only areas covered for more than five consecutive days are included. Though the reflectivity of snow fields as well as bare surfaces varies within a wide range, the establishment and disappearance of snow introduces by far the most striking seasonal change into surface albedo¹⁵. R . The parametrised measure of radiation (langley d^{-1}) reflected from snow and ice in excess of that which would be

reflected in the same situation by bare ground or ocean free of ice cover.

The principal advantage of the QTS-1 model with immobilised atmosphere and non-existent heat storage capacity is that local air surface temperatures are a function of instantaneous local irradiation and albedo only. Thus, solution of our problem, which is to locate the sensitive zone and season, can be found by determining where and when the observed real world behaviour most closely approaches the relationship anticipated in the model.

Interpretation of QTS variations

The observed data are in Table 1. The globe is warmest in July under low Q and coldest in January under high Q . The global mean temperature is not a direct function of global mean Q , and the seasonal Q wave is approximately symmetrical between the two hemispheres whereas the T wave is not.

Figure 1 shows the variation of global and hemispheric mean T compared to areal changes of T_0 , S , Q_0 , Q_{200} , and Q_{400} . The T_0 area closely approaches the S area, so that for the purpose of the present discussion, T_0 can be considered equal to S . There is an inverse relationship between the global T and S ; in a first order approximation, the global mean T and thus the global mean climatic state could be considered a function of S . The same relationship is valid for the Northern Hemisphere.

The seasonal wave of global T and S is shaped in agreement with the T and S waves in the Northern Hemisphere because of high seasonal contrast over the northern continents. Thus, the seasonal variation of temporal global climate states seems to be basically controlled by variations in snow and ice cover area in the Northern Hemisphere.

The fast global increase of S between September and November coincides with the buildup of snow and with the increase of Q_{200} area in the Northern Hemisphere, and with the sharp increase of R in the Southern Hemisphere, when the planet passes from low surface reflection (LSR) to high surface reflection (HSR) mode. Although the increase of S in the Northern Hemisphere is relatively fast and the decrease slow, the opposite seems to hold true for the Southern Hemisphere where the buildup of fields of pack ice is slowed down by the heat reserves in the ocean. The snow cover, once established with its high albedo and high specific heat required for melting has a self-perpetuating cooling effect on environment.

In Fig. 2 the rates of change in S and Q_{200} areas are plotted

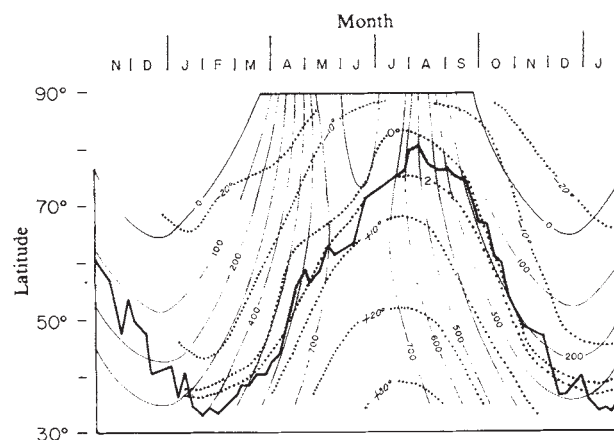


Fig. 3 Seasonally changing mean latitudinal position of the snowline (full black line) from October 1971, until January 1973, between $50^{\circ}E$ and $70^{\circ}E$ compared with long term mean surface air temperatures ($^{\circ}C$) (dotted lines: zone between $0^{\circ}C$ and $+2^{\circ}C$ stippled) and ground level cloud free insolation Q (langley d^{-1}). From September through November the snowline and the $0^{\circ}C$ isotherm follow the 200 langley d^{-1} isoline. First day of each month is marked.

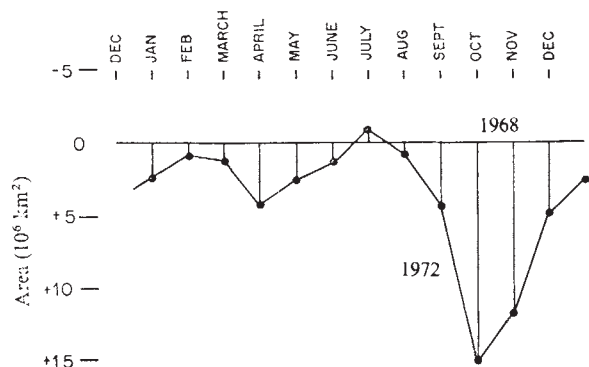


Fig. 4 Departure of the monthly mean area covered by snow and ice in the Northern Hemisphere in 1972 (cold anomaly) from 1968 levels.

as a function of season. The ΔQ_{200} values are almost the same in both hemispheres whereas the ΔS is greatest in October and November in the Northern Hemisphere. The disintegration of snow fields in the Northern Hemisphere and pack ice in the Southern Hemisphere are substantially slower.

The bottom line in Fig. 2 shows the sensitivity index (SI) which indicates the relationship between simultaneous ΔQ_{200} and ΔS . Index values are irrational and are not plotted (denoted by connecting dotted line) if ΔQ_{200} or ΔS approach zero. Large SI indicates that the rate of change of Q_{200} closely parallels that of the S areas and thus the high sensitivity of S to insolation change. The greatest SI for the globe (about 60), Northern Hemisphere (about 80) and the Southern Hemisphere (about 13) are reached simultaneously in October and November, identifying this interval as the sensitive season.

Changing mean latitudinal position of the snowline in the interior of the Euroasian continent and the corresponding T and Q values are shown in Fig. 3. They were plotted for the meridional segment between 50° E and 70° E, which is composed mainly of lowland. The advancing snowline in autumn, together with the T_0 boundary, closely follow the isoline with irradiation $Q = 200$ langley d^{-1} .

In contrast, between March and June, during the poleward retreat of T_0 and S , the snowline move is substantially delayed relative to Q and the irradiation above the snowline steadily increases from 400 to about 800 langley d^{-1} . Thus, in the studied segment, a distinct seasonal asymmetry exists in the relationship of Q to S . The snowline essentially follows the irradiation drop in autumn but it is substantially delayed behind insolation rise in spring¹⁷.

Between September 1 and December 21 in the North American and Euroasian interiors, the Q at the snowline drops only by about 2 langley d^{-1} for each degree of latitude of snow fields southward advance. Q increases by about 15 langley d^{-1} (from 10–22) per degree of latitude of snowline retreat between March 1 and June 21. Similar measurements in the Southern Hemisphere give the Q departure at the advancing pack ice front as about 45 langley d^{-1} degree⁻¹. The greatest sensitivity of S to Q (and thus of climate to Q) is reached at the time when the snowline accurately follows the shifting insolation pattern resulting in zero change in Q over the snowline. The conditions observed during autumn in the interior of the continents of the northern hemisphere are closest to this situation.

Test against present weather variability

In the real world the surface temperature and reflectivity depend on a number of factors, many of them unrelated to local insolation. Thus, simple relationships anticipated in the model may not exist at all. On the contrary, feedbacks may exist strengthening the dependence.

There are several ways the validity of tentative conclusions derived from the $QTS-1$ model could be tested. One is mathematical modelling which could be undertaken in the future. Others are various partial tests, using the present observed

short term fluctuations in meteorological variables. Such tests could include correlation of past snow cover history with annual temperatures. The present hypothesis would be supported if significantly high correlation was found between the years with relatively extensive snow cover in fall and the anomalously cold years. Satellite observations of snow as yet cover too little time to provide statistically significant tests. Available maps, however, include four relatively normal years (1967–70) and two anomalous cold years, 1971 and 1972. The atmosphere below the 500 mbar pressure level north of 15° N was 0.35° C cooler in 1972 than in 1968 (ref. 18). So, at least for the northern hemisphere, the snow and ice cover extent of a 'normal' year could be compared with that corresponding to the anomalously cold year. Figure 4 demonstrates that the 1972 anomaly, as compared with 1968, was indeed characterised by significantly larger area of snow and ice on an annual average and by significantly earlier deposition of snow in autumn.

Test against palaeoclimatic history

If the continents in the northern high and mid-latitudes are the sensitive area, and the autumn is the sensitive season intercepting the extraterrestrial impulses, then the timing of the expected impact of Milankovitch's mechanism on this area and season should fit the radiometrically dated palaeoclimatic evidence.

Figure 5 shows the seasonal variation over the past 30,000 yr in energy flux at the top of the atmosphere in the plane facing the Sun, as a result of the Milankovitch mechanism. The September and October mean global irradiation on the top of the atmosphere peaked about 6,000 yr ago, reaching 725 langley d^{-1} and dropped to a minimum of 675 langley d^{-1} about 17,000 yr ago, with corresponding change of Q values.

It is well known from isotopically dated palaeoclimatic evidence that the highest postglacial surface temperatures were reached about 5,000–7,000 yr ago and that the most recent glacial culminated sometime between 15,000 and 19,000 yr ago¹⁹. In a first approximation the correlation can be extended to at least 150,000 yr BP simply by using conventional Milankovitch summer or winter half-year curves with timing shifted by 5,000 yr (ref. 20).

Within the precision limits of radiometric dating the gross shape of palaeoclimate curves for the past 20,000 yr also correlates well with the irradiation levels in early autumn^{4,19,20}. This observation, even though not yet analysed quantitatively,

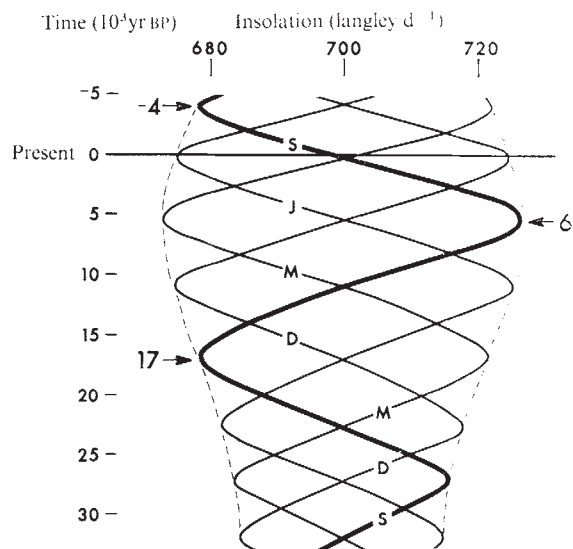


Fig. 5 Irradiation at the top of the atmosphere in the plane perpendicular to solar rays during the past 30,000 yr, at the summer solstice (J), winter solstice (D) spring equinox (M) and autumn equinox (S). According to my $QTS-1$ model, the autumn irradiation, approximately represented by the S curve, controls the global extent of cryosphere and through it the climate.

lends substantial support to my hypothesis, which predicts delayed snow accumulation in the Northern Hemisphere under the high autumn irradiation 6, 28, 42, 55, 77, 101 and 122 thousand years ago with, by contrast, an early snow accumulation in the north and a late pack ice melting in the south under the low early fall irradiation 17, 36, 51, 67, 89, 111 and 134 thousand years ago. It could be speculated that the mechanism was less efficient during full glacial conditions in North America because of substantially reduced seasonal snow fields at the expense of permanent ice. The opposite holds true for Asia and Eastern Europe. So the North American subcontinent could have been less sensitive and Asia more sensitive to insolation change during a full glacial than they are today. Also the timing of the most sensitive interval may have shifted between August and November according to the varying surface properties of the Pleistocene continents.

Because the QTS-1 model does not recognise heat and water transfer in the atmosphere, the validity of the conclusions in the real world remains tentative. But my tentative results indicate that (1) the conventional assumption on summer half-year insolation in the northern hemisphere as linking Milankovitch's mechanism with climate through millenia long delay is probably invalid, (2) an accurate astronomical dating of gross Pleistocene climatic history is feasible since the climate probably adjusts to changing insolation patterns with negligible delay, (3) the long term outlook for natural climate development as predicted from the QTS-1 model is the perpetuation of oscillatory cooling for another 4,000 yr and (4) the heat balance developments in the inner parts of the

northern continents deserve the close attention of long-range weather forecasters. The sensitivity concept provides a plausible explanation of the 20,000-yr climate cycle and a new look at the mechanism of climatic variability. Nevertheless it is far from a complete theory of climate change and leaves unexplained the causes of the 10⁶ yr Pleistocene glacial cycle as well as the causes of climatic variability on the time scales of millenia.

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Darwinian evolution in the genealogy of haemoglobin

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Frequencies of mutations between reconstructed ancestor and descendant sequences of codons for metazoan globin chains show that natural selection guided protein evolution. Mutations which improved haemoglobin function were accepted at an accelerated rate in the first vertebrates. Rates decelerated after functional opportunities had been exploited.

THE differences between 55 contemporary globins with well analysed amino acid sequences¹⁻⁴⁷ have been used to deduce the genealogical tree shown in Fig. 1. Analysis of patterns of mutation between ancestors and descendants on this tree shows that natural selection improved the haemoglobin molecule and then preserved the improvements. Early evolution proceeded at a faster rate (mutational replacements per unit time) than later evolution. This can be shown to correlate with Darwinian theory by taking advantage of the identification of functional residues (Table 1) by Perutz and coworkers⁴⁸⁻⁵³. We have deduced that during the evolutionary transition of monomeric haemoglobin to a tetramer with a sigmoid oxygen equilibrium curve the residue positions which acquired cooperative functions changed more rapidly than other positions. Such a pattern cannot be explained by the theory of random fixation of

selectively neutral mutations⁵⁴, so called non-Darwinian evolution⁵⁵. It can be attributed, however, to positive selection for more optimal function.

Reconstruction of genealogy

A maximum parsimony approach^{56,57} was used to reconstruct the genealogy of the globin chains. According to the parsimony principle⁵⁶⁻⁶², the best genealogy requires the fewest possible nucleotide replacements. In such a genealogy the maximum number of sequence similarities is associated with common ancestry, and the minimum number with parallel and back changes.

A mathematically rigorous procedure reconstructs the most parsimonious ancestors for a given genealogy⁵⁶. There is no known method, short of an exhaustive search, for finding the most parsimonious genealogy. Thus we used an heuristic approach, in which an initial, unweighted pair-group cluster analysis⁶³ was improved repeatedly by a branch-swapping procedure⁵⁷. The search was extended by sliding portions of the globin alignment in order to further reduce the length (that is total number of nucleotide replacements) of the genealogy.

Regions in the genealogy in which few nodal points span long periods of time were underrepresented with respect to nucleotide replacements. Thus we compensated for lost

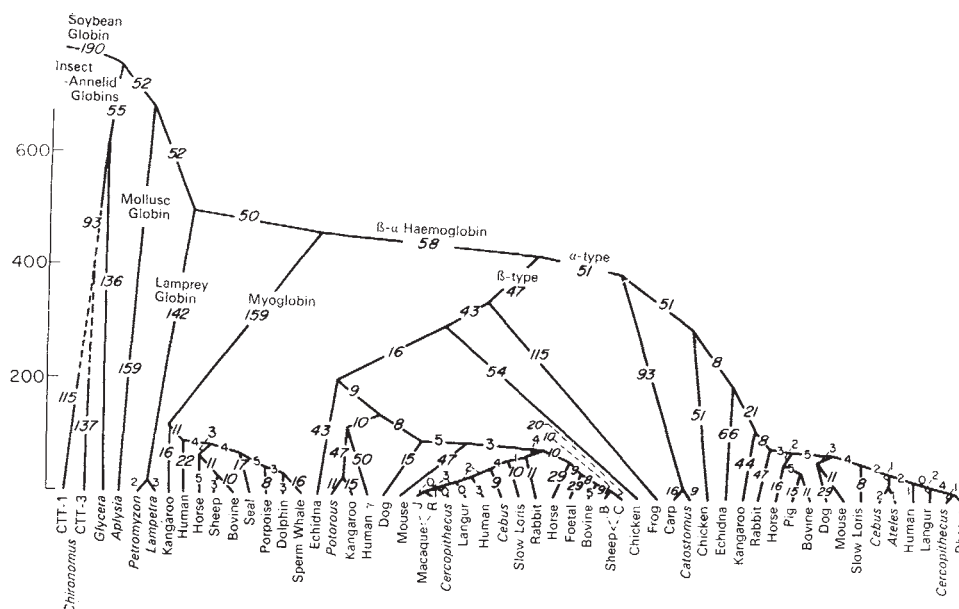


Fig. 1 Globin genealogical tree. Soybean¹, insect CTT-1 (Braunitzer, unpublished) and CTT-3 (ref. 2) (*Chironomus thummi*), annelid (*Glycera dibranchiata*)³, mollusc (*Aplysia limacina*)⁴, *Petromyzon marinus*⁵, *Lampetra fluviatilis*⁶, myoglobin of kangaroo (*Megaleia rufa*)⁷, human⁸, horse⁹, sheep¹⁰, bovine¹¹, harbour seal (*Phoca vitulina*)¹², porpoise (*Phocaena phocaena*)¹³, dolphin (*Delphinus delphis*)¹⁴, sperm whale (*Physeter catodon*)¹⁵, β echidna (*Tachyglossus aculeatus*)¹⁶, β *Potorous tridactylus*¹⁷, β kangaroo (*Macropus giganteus*)¹⁸, γ human¹⁹, β dog²⁰, β mouse C57BL²¹, β Japanese macaque (*Macaca fuscata*)²², β rhesus (*Macaca mulatta*)²³, β *Cercopithecus aethiops*²⁴, β langur (*Presbytis entellus*)²⁵, β human²⁶, β *Cebus apella*²⁷, β slow loris (*Nycticebus coucang*)²⁸, β rabbit²⁹, β horse^{30,31}, foetal β bovine³², β bovine B^{31,33}, β sheep B^{31,34} and C^{31,34}, β chicken³⁵, β frog (*Rana esculenta*)³⁶, α carp (*Cyprinus carpio*)³⁷, α *Catostomus clarkii*³⁸, α chicken³⁹, α echidna (*Tachyglossus aculeatus*)⁴⁰, α kangaroo (*Macropus giganteus*)⁴¹, α rabbit^{31,42}, α slow horse⁴³, α pig^{31,44}, α bovine⁴⁵, α dog²⁰, α mouse C57BL⁴⁶, α slow loris (*Nycticebus coucang*)²⁸, α *Cebus apella*²⁷, α *Ateles geoffroyi*⁴⁷, α human²⁶, α langur (*Presbytis entellus*)²⁵, α *Cercopithecus aethiops*²⁴ and α rhesus (*Macaca mulatta*)²³. Link lengths are the numbers of nucleotide replacements between adjacent ancestor and descendant sequences and are italicised when our augmentation algorithm corrects for superimposed replacements. Original and corresponding augmented link lengths are as follows: 0-0, 1-1, 2-2, 3-3, 4-4, 5-5; 6-8, 7-9, 8-10, 9-11; 10-15, 11-16, 12-17; 14-20, 15-21, 16-22; 18-29; 20-43, 21-44, 24-47, 27-50, 28-51, 29-52, 31-54, 32-55; 34-58; 36-66; 50-93; 59-115; 63-136, 64-137; 68-142, 85-159; 90-190. The ordinate is a time scale in Myr based on palaeontological views concerning the ancestral separations of the organisms from which the globins came.

nucleotide replacements in these underrepresented regions by an augmentation procedure⁵⁷ based on the distribution of nucleotide replacements in densely sequenced regions. The results obtained by our algorithm are highly correlated with results obtained by the stochastic method using Poisson distribution^{64,65} for estimating hidden mutational change in codons (G. W. M., M. G., and R. Holmquist unpublished).

Myoglobin-haemoglobin separation

In the genealogy and alignments found earlier⁶⁶ the line from the ancestral metazoan globin to the vertebrate β-α haemoglobin ancestor gave off in succession annelid, insect, mollusc, lamprey and mammalian myoglobin branches. Its length was 1,636 nucleotide replacements. Our current genealogy (Fig. 1) with its corresponding alignments requires 1,629 replacements. It joins annelid and insect branches, but otherwise retains the same topology. The ancestral separation between mammalian myoglobin and β-α haemoglobin branches still occurs after the lamprey separation. To change the position of the mammalian myoglobin branch costs too many additional mutations. (The aligned sequences of the 55 contemporary globins and the reconstructed codons for these sequences as well as for the 53 ancestral nodes may be obtained by request to the authors.)

Non-uniform globin evolution

The numbers on the links in Fig. 1 are the nucleotide replacement values separating adjacent ancestral and descendant sequences. Replacement values corrected for superimposed mutations are italicised. These values were used in Table 2 for calculating the rates of globin evolution during different evolutionary periods. The estimated time scale is based on palaeontological views⁶⁷⁻⁷⁰, some of which have to be considered educated guesses. Perhaps the most uncertainty pertains

to the points for the lamprey-jawed vertebrate and invertebrate-vertebrate dichotomies. The times chosen of 500 Myr ago for the former and 680 Myr ago for the latter are more ancient than actually documented in the fossil record. These dichotomies are, however, not less than 420 and 540 Myr old, respectively⁷¹. By the same token, the fossil record of the late Precambrian, while revealing unicellular organisms in abundance, provides no hint of multicellular life until very close to the end of this epoch⁷²⁻⁷⁴. Thus the ancestral separation of invertebrates (molluscs, insects, annelids) and chordates was not likely to have been earlier than 650-700 Myr ago, and it could have been somewhat later.

On *a priori* grounds⁷⁵⁻⁷⁹ and from results of Table 2, we think that rapid rates followed by slow rates occurred in globin evolution—first, when selection shaped the prototypic globin, then when new functions emerged in members of the globin family. The most ancestral portion of the reconstructed genealogy is too sparsely populated to test this hypothesis adequately. Thus Table 2 starts from the invertebrate-vertebrate ancestor at 680 Myr ago, and focuses on the vertebrates. They encompass the denser region of the genealogy and are more readily dated by palaeontological evidence. Rates are in nucleotide replacements per 100 codons per 10⁸ yr (NR%). During the span from the late Precambrian to the present, the globin genes evolved at an average rate of 31 NR%. This is more than three times faster than the rates found in studies^{78,80,81} in which distances between the contemporary sequences were not corrected sufficiently for superimposed mutations. Moreover, in contradistinction to conclusions⁸² on constancy of evolutionary rates, the haemoglobin genes evolved at markedly non-constant rates. For the first 380 or so Myr from the invertebrate-vertebrate to the amniote (chicken-mammal) ancestor, the haemoglobin genes evolved at an average rate of 46 NR%, but for the next 300 Myr from the amniote

ancestor to the present they evolved at the average rate of only 15 NR%. Comparable values for the unaugmented lengths are 25 NR% in the earlier period and 11 NR% in the later period. This difference is supported by a χ^2 analysis, which uses the above time estimates and the uniform rate assumption as the null hypothesis (augmented: $\chi^2 = 347.3043$, $P < 0.001$, 1 d.f.; unaugmented: $\chi^2 = 102.8298$, $P < 0.001$, 1 d.f.). Non-uniform globin evolution has also been observed by others⁸³.

As Table 2 shows, globin evolution accelerated in the early vertebrates. This acceleration correlates with gene duplications, which first separated the vertebrate myoglobin and haemoglobin branches and then subdivided the haemoglobin branch into α and β lineages. From 500 to 400 Myr ago the genes descending from the basal vertebrate ancestor through the haemoglobin-myoglobin and β - α ancestors to the teleost-tetrapod α ancestor evolved at the rate of 109 NR%. For the following 100 Myr between the teleost-tetrapod and amniote ancestors, the rate of α evolution slowed to 36 NR%, and then over the next 300 Myr (to the present) to an average rate of 14.5 NR%. When the vertebrate haemoglobin genes are traced from the 500 Myr old vertebrate ancestor to the tetrapod β ancestor of about 340 Myr ago, the rate of nucleotide replacements comes out to 66 NR%. As the rate was 109 NR% for the genes traced to the 400 Myr old teleost-tetrapod α ancestor,

β genes at first must have evolved less rapidly than α genes. Between the tetrapod and amniote β ancestors, the β rate accelerated to 74 NR%, but then β evolution, like α evolution, abruptly slowed. The different lines of β descent from the amniote ancestor to the present evolved at an average rate of only 16 NR%.

Selectionist interpretation

We interpret the initial acceleration-eventual deceleration pattern in protein evolution this way. When mutations first produce a potentially useful protein, the possibilities for improvements are considerable. Thus mutations in the gene coding for the protein will often be advantageous and selected for, but after the protein has been perfected, a much larger proportion of mutations would be detrimental and selected against. Once many proteins have become optimised, that is more intricately coadapted for the cooperative execution of their functions, macromolecular evolution would slow. Such a pattern, however, could be temporarily interrupted by periods of accelerated evolution. This happened in early vertebrates. Mutations were selected in duplicated genes which multiplied the number of proteins with differentiated functions. Accelerated change occurred while selection was optimising the structures of these new proteins, but eventually the stabilising component of selection slowed the further rate of change.

Table 1 Residue positions with defined functional roles in mammalian α and β haemoglobin chains

Helical position	β functions	α functions	Helical position	β functions	α functions
	Haem contact sites			Non-Bohr-associated, $\alpha_1\beta_1$ contact sites	
B13	HC, IP	HC, IP	B11		$\alpha_1\beta_1$
C4	HC, IP	HC, IP	B12		$\alpha_1\beta_1$
C7	HC	HC, $\alpha_1\beta_2$	B15	$\alpha_1\beta_1$	$\alpha_1\beta_1$
CD1	HC, IP	HC, IP	B16	$\alpha_1\beta_1$	$\alpha_1\beta_1$
CD3	HC	HC	C1		$\alpha_1\beta_1$
CD4	HC, IP	HC, IP	D2	$\alpha_1\beta_1$	
E7	HC	HC	D6	$\alpha_1\beta_1$	
E10	HC		G10	$\alpha_1\beta_1$	$\alpha_1\beta_1$
E11	HC, IP	HC, IP	G11		$\alpha_1\beta_1$, IP
E14	HC		G13		$\alpha_1\beta_1$
E15	HC, IP		G14	$\alpha_1\beta_1$	$\alpha_1\beta_1$
F4	HC	HC	G17	$\alpha_1\beta_1$	
F7	HC	HC	G18	$\alpha_1\beta_1$	$\alpha_1\beta_1$
F8	HC	HC	GH2	$\alpha_1\beta_1$	$\alpha_1\beta_1$
FG3	HC	HC, $\alpha_1\beta_2$	GH5	$\alpha_1\beta_1$	$\alpha_1\beta_1$
FG5	HC, $\alpha_1\beta_2$, IP	HC, $\alpha_1\beta_2$, IP	H1	$\alpha_1\beta_1$	
G4	HC, $\alpha_1\beta_2$	HC	H2	$\alpha_1\beta_1$	$\alpha_1\beta_1$
G5	HC, IP	HC, IP	H3	$\alpha_1\beta_1$	
G8	HC, IP	HC, IP	H5	$\alpha_1\beta_1$	
H12		HC, IP	H6	$\alpha_1\beta_1$	$\alpha_1\beta_1$
H15	HC, IP	HC, IP	H9	$\alpha_1\beta_1$	
H19	HC, IP	HC, IP			
	Non-haem, $\alpha_1\beta_2$ contact sites			Remaining interior positions (stabilises tertiary structure)	
C2	$\alpha_1\beta_2$	$\alpha_1\beta_2$	A8	IP	IP
C3	$\alpha_1\beta_2$	$\alpha_1\beta_2$	A11	IP	IP
C5	$\alpha_1\beta_2$	$\alpha_1\beta_2$, BE	A12	IP	IP
C6	$\alpha_1\beta_2$	$\alpha_1\beta_2$	A15	IP	IP
CD2	$\alpha_1\beta_2$	$\alpha_1\beta_2$	B6	IP	IP
FG4	$\alpha_1\beta_2$	$\alpha_1\beta_2$	B9	IP	IP
G1	$\alpha_1\beta_2$	$\alpha_1\beta_2$	B10	IP	IP
G2	$\alpha_1\beta_2$	$\alpha_1\beta_2$	B14	IP	IP
G3	$\alpha_1\beta_2$	$\alpha_1\beta_2$	D5	IP	
HC2	$\alpha_1\beta_2$, IBE, IP	$\alpha_1\beta_2$, IBE, IP	E4	IP	IP
HC3	$\alpha_1\beta_2$, $\beta\beta$, BE		E8	IP	IP
Non- $\alpha_1\beta_2$, like-chain contact, Bohr effect, and 2,3-DPG binding sites			E12	IP	IP
NA1	$\beta\beta$, DPG	$\alpha\alpha$, BE	E15		IP
NA2	$\beta\beta$, DPG		E18	IP	IP
A4		$\alpha\alpha$, IBE	E19	IP	IP
B12	IBE, $\alpha_1\beta_1$		F1	IP	IP
C1	IBE, $\alpha_1\beta_1$		G11	IP	
EF6	DPG		G12	IP	IP
FG1	BE		G16	IP	IP
G6		$\alpha\alpha$	H8	IP	IP
H5		IBE, $\alpha_1\beta_1$	H11	IP	IP
H9		$\alpha\alpha$, BE, $\alpha_1\beta_1$	H12	IP	
H10	$\beta\beta$	$\alpha\alpha$, IBE			
H21	$\beta\beta$, DPG				
HC3		$\alpha\alpha$, BE			

HC, Haem contact; IP, interior position; $\alpha_1\beta_2$, $\alpha_1\beta_2$ contact; BE, Bohr effect site; IBE, interacts with Bohr effect site; $\beta\beta$, $\beta\beta$ contact; $\alpha\alpha$, $\alpha\alpha$ contact; DPG, 2,3-diphosphoglycerate binding site; and $\alpha_1\beta_1$, $\alpha_1\beta_1$ contact.

We assume that homotetramers preceded heterotetramers in haemoglobin evolution. This would explain why the amino acids involved in interchain contacts occur in α and β chains mostly at homologous residue positions (Table 1). We assume further that the ancestral homotetramer had subunits more β -like in polymer forming properties than α -like, because homotetramers are readily formed by β chains but not by α ^{84,85}. Many invertebrate haemoglobins are monomers. Lamprey haemoglobin chains in the oxygenated state are also monomers. But in the deoxy state, they form homodimers⁸⁶ and transitory tetramers⁸⁷. Thus a haemoglobin more complicated than a simple monomer probably existed in the earliest vertebrates, as lampreys belong to the most primitive vertebrate class. This first vertebrate haemoglobin, if it behaved like lamprey haemoglobin, released oxygen from its haem iron atoms more readily at reduced pH and in its aggregated state. It would have been, therefore, a more useful protein than the homotetramers of β chains which lack even the rudiments of cooperativity^{84,85}. Natural selection could then exploit the possibilities for bringing about a haemoglobin more efficient at delivering oxygen to the tissues of larger-bodied, fast-moving animals. This required the evolution of heterotetrameric haemoglobin.

After the β - α gene duplication in a common ancestor of teleosts and tetrapods, positive Darwinian selection acted on the nascent α locus while stabilising selection was stronger at the β locus. Once the older β_4 type homotetramer was, however, replaced by the heterotetramer with a specialised α chain, positive selection for a more differentiated β chain intensified. This caused the rate of β evolution to accelerate between the tetrapod and amniote ancestors. Functionally superior tetrameric haemoglobin emerged. Then α and β loci were both subjected to intense stabilising selection. Evidence for this selectionist interpretation is provided by the following more detailed analysis.

Positive selection for improved function

The residue positions in Table 1 are arranged according to their functions in present day mammalian α and β chains. The positions with the most crucial functions are first the haem contacts and second those concerned with the interchain

cooperativity which facilitates oxygen delivery, the $\alpha_1\beta_2$ contacts and the salt-bridge-forming sites associated with the Bohr effect, respectively. There are many nucleotide replacements between the mollusc-vertebrate globin and amniote α and β ancestors (Table 3) at these positions, but almost no changes in the amniotes (Tables 4 and 5).

Table 3 shows the replacement values from the invertebrate-vertebrate ancestor through the basal vertebrate and the myoglobin-haemoglobin and β - α ancestors to the basal amniote α and β sequences. The most slowly evolving positions are those which now function in both α and β haemoglobin chains as haem contacts, and which must have been well established as such as far back as 600–700 Myr ago. Yet these haem contacts, although conservative compared with other residue positions, show a seven times faster evolutionary rate in the preamniotes than in the amniotes (compare Tables 3, 4, and 5). Presumably, haem-haem cooperative interactions were not fully perfected until about 300 Myr ago.

Positive Darwinian selection in the emerging vertebrates is indicated by the high proportion of changes at positions which now function as $\alpha_1\beta_2$ contacts in both α and β chains. These contacts favour haem-haem interactions by placing the haems of the contacting chains in close proximity. Positions which acquired this function evolved four times faster than the average rate over all positions (Table 3). Mutations occurred at C5, C6, FG4, G1 and HC2, five of the nine positions which now function in both α and β chains as $\alpha_1\beta_2$ contacts, as well as at FG3, a haem contact which also functions as an $\alpha_1\beta_2$ contact in amniote α chains. The mutations of glutamic acid (GAG) to glutamine (CAG) at C5, valine (GUG) to glutamine (CAG) at FG4, asparagine (AAU) to aspartic acid (GAU) at G1, isoleucine (AUU) to tyrosine (UAU) at HC2, and arginine (CGU) to leucine (CUU) at FG3 persisted. They are central to the interchain cooperativity of present-day tetrameric haemoglobin.

Between the myoglobin-haemoglobin and β - α ancestors, the sites which now function as $\alpha_1\beta_1$ contacts in both α and β chains evolved at twice the average rate of all positions (Table 3). Lysine (AAA) mutated to isoleucine (AUA) at B15, aspartic acid (GAU) to asparagine (AAU) at G10, aspartic acid (GAU)

Table 2 Rates of mollusc and vertebrate globin evolution

Evolutionary period	Age (Myr BP) Mollusc, agnathan, and jawed vertebrate globins	Nucleotide replacements per 100 codons per 10 ⁸ yr
Invertebrate-vertebrate ancestor to <i>Aplysia</i> 'myoglobin'	680– 0	16.1
Invertebrate-vertebrate ancestor to vertebrate globins	680– 0	31.2 (20.5–35.3)
Invertebrate-vertebrate to vertebrate ancestor	680–500	19.5
Vertebrate ancestor to lamprey globins	500– 0	21.0 (20.9–21.0)
Vertebrate ancestor to myoglobins	500– 0	31.6 (29.6–33.2)
Vertebrate ancestor to α_s and β_s	500– 0	37.1 (33.5–40.9)
Vertebrate to therian myoglobin ancestor	500–120	36.2
Therian myoglobin ancestor to myoglobins	120– 0	17.2 (8.7–23.4)
Vertebrate to tetrapod β ancestor	500–340	65.7
Tetrapod β ancestor to β_s	340– 0	22.9 (18.3–28.4)
Vertebrate to teleost-tetrapod α ancestor	500–400	108.9
Teleost-tetrapod α ancestor to α_s	400– 0	19.9 (18.1–23.9)
α Lineage		
Teleost-tetrapod ancestor to teleost α_s	400– 0	18.7 (18.1–19.3)
Teleost-tetrapod ancestor to amniote α_s	400– 0	20.0 (18.6–23.9)
Teleost-tetrapod to amniote α ancestor	400–300	36.2
Amniote ancestor to chicken α	300– 0	12.1
Amniote ancestor to mammalian α_s	300– 0	14.7 (12.5–19.9)
Amniote to eutherian α ancestor	300– 90	12.5
Eutherian ancestor to human α	90– 0	13.4
Eutherian ancestor to all eutherian α_s	90– 0	18.5 (12.6–37.0)
β Lineage		
Tetrapod ancestor to frog β	340– 0	24.2
Tetrapod ancestor to amniote β_s	340– 0	22.8 (18.3–28.4)
Tetrapod to amniote β ancestor	340–300	73.6
Amniote ancestor to chicken β	300– 0	12.3
Amniote ancestor to mammalian β_s	300– 0	16.3 (12.3–22.3)
Amniote to eutherian β ancestor	300– 90	10.8
Eutherian ancestor to human β	90– 0	16.7
Eutherian ancestor to all eutherian β_s	90– 0	26.6 (11.4–49.4)

Table 3 Nucleotide replacement lengths for groups of residue positions

Type of residue positions	During emergence of hypothetical homopolymer						During evolution of nascent heterotetramer			
	Invert-vert. to vert. anc.		Vert. to myo.-Hb anc.		Myo-Hb to β - α anc.		β - α to amniote α anc.		β - α to amniote β anc.	
	No. of positions	Average length	No. of positions	Average length	No. of positions	Average length	No. of positions	Average length	No. of positions	Average length
Haem contacts in both β and α and in myoglobin chains	18	0.11	18	0.17	18	0.06	18	0.17	18	0.11
Non-haem $\alpha_1\beta_2$ contacts in both β and α chains	9	0.78	9	0.00	9	0.22	9	0.67	9	0.44
Like-chain contacts in both β and α chains	3	0.00	3	0.00	3	0.00	3	0.33	3	0.67
$\alpha_1\beta_1$ contacts in both β and α chains	13	0.23	13	0.23	13	0.38	13	0.31	13	0.31
With defined functions in α but not β chains	7	0.14	7	0.14	7	0.29	7	0.86	7	0.14
With defined functions in β but not α chains	12	0.33	12	0.08	12	0.17	9	0.11	12	0.67
Remaining interior positions	19	0.16	19	0.26	19	0.05	18	0.22	19	0.26
Remaining exterior positions	67	0.13	67	0.21	67	0.31	64.5	0.48	65	0.28
All positions	148	0.20	148	0.18	148	0.23	141.5	0.40	146	0.30

Positions are grouped according to their functional roles in present-day haemoglobin chains, as may be identified from Table 1; unaugmented nucleotide replacements for the positions in each group during each period of descent are averaged. B12, C1, H5, and H9 are included in the group consisting of $\alpha_1\beta_1$ contacts in both β and α chains since the emergence of this function probably preceded the emergence of their Bohr-effect-related function. The seven positions with defined functions in α but not β chains are A4, B11, CD2, G6, G11, G13, and H12. The twelve positions with defined functions in β but not α chains are NA2, D2, D6, E10, E14, E15, EF6, FG1, G17, H1, H3 and H21.

to valine (GUU) at G14, alanine (GCG) to proline (CCG) at GH2, and also alanine (GCU) to proline (CCU) at H2. Then these amino acids, along with those at other prospective $\alpha_1\beta_1$ contact sites, either persisted or mutated to similar amino acids in later amniote haemoglobin chains. This supports the notion that the β - α gene was coding for homotetrameric haemoglobin even before it duplicated. Conversely in the myoglobin lineage, tyrosine mutated to histidine, tyrosine to lysine, leucine to histidine, aspartic acid to proline, proline to valine, and glutamic acid to lysine at C1, C7, FG3, G1, G2, and H9, respectively. These mutations at positions which function as interchain contacts in haemoglobin polymers helped reshape the myoglobin molecule into a high oxygen affinity monomer.

The positions which are $\alpha_1\beta_2$ contacts in the two haemoglobin chain types evolved at a faster than average rate in the nascent α and β lineages (Table 3). Also the positions categorised as like-chain contacts in both α and β chains evolved at a faster than average rate because of mutations at HC3, a Bohr effect site in both α and β chains, as well as an $\alpha_1\beta_2$ contact site in β chains. (Ancestral lysine (AAG) mutated to arginine (AGG) at α HC3 and to histidine (CAU) at β HC3.) Positions which acquired important functions in α chains but not in β evolved in the α lineage at a faster rate than other positions. Similarly, positions which acquired important functions in β chains but not in α evolved in the β lineage at a faster rate than other positions.

The evidence in Table 3 for positive selection directing the evolution of early vertebrate haemoglobin is supported by a χ^2 test, performed to test the null hypothesis that sites where mutations produced acquired crucial functions did not evolve at a different rate from all other sites. Homopolymer emergence was tested by comparing nucleotide replacements at prospective $\alpha_1\beta_2$ and $\alpha_1\beta_1$ contact positions with all other positions in the invertebrate-vertebrate to vertebrate and myoglobin-haemoglobin to β - α ancestors ($\chi^2 = 7.312$, $P < 0.01$, 1 d.f.). Heterotetramer evolution was tested by comparing nucleotide replacements at $\alpha_1\beta_2$ contacts, like-chain contacts, and α -only functions in the β - α to amniote α lineage, plus $\alpha_1\beta_2$ contacts, like-chain contacts, and β -only functions in the β - α to amniote β lineage, with the other positions ($\chi^2 = 11.403$, $P < 0.001$, 1 d.f.).

Mutations were fixed in the diverging α and β genes because they produced functionally superior haemoglobin molecules. By the time amniote tetrapods evolved, heterotetramers with modern allosteric properties had replaced the older models.

Thereafter natural selection acted against further amino acid substitutions at the residue positions involved in haem and $\alpha_1\beta_2$ contacts and the Bohr effect. Nucleotide replacements occurred in the codons for these positions at only one-fifth to one-tenth the average rate (Tables 4 and 5). Moreover, the average rate itself was much reduced.

Darwinian selection, however, was still directing haemoglobin evolution. This is demonstrated by nucleotide replacements at 2,3-diphosphoglycerate (DPG)-binding sites. For example, between the amniote and mammal (protherian and therian) β ancestor, glutamine (CAG) at H21 mutated to histidine (CAU). The greater binding of DPG to the β chain produced by this mutation further facilitated the unloading of oxygen from haemoglobin in the deoxy conformation^{51,53}. The effect was reversed in the line to human γ chain by H21 histidine mutating to serine. This mutation favoured the ability of foetal haemoglobin to compete with adult haemoglobin for oxygen.

Darwinian selection can also explain changes observed at positions NA2, A2, A7, CD2, D2, E2, EF5, G18, GH2 and H2 in descent from the metazoan ancestor to human α and β sequences; proline replaced alanine five times more often than alanine replaced proline. Alanines can appear anywhere in a globin chain, but prolines are restricted to sites at the end of helices or to non-helical regions where they delimit the lengths

Table 4 Effect of functions on nucleotide replacement lengths in the amniote ancestor-to-contemporary α lineages

Type of residue positions	No. of positions	Average length	Nucleotide replacements per position per 100 Myr
Haem contacts	19	0.37	0.02
Non-haem contact $\alpha_1\beta_2$ contacts	10	0.20	0.01
Salt bridges, associated Bohr effect, $\alpha\alpha$ contact	7	0.14	0.01
Non-salt bridge $\alpha_1\beta_1$ contacts	14	2.00	0.13
Remaining interior positions	19	1.47	0.09
Remaining exterior positions	72	2.38	0.15
All positions	141	1.68	0.11

Positions are grouped according to their functional roles, as may be identified in Table 1; unaugmented nucleotide replacements over the amniote (chicken-mammal) α region of the globin genealogy are averaged for the positions in each group. The times of the connecting links over the amniote region, when added up, represent about 1.6×10^9 yr.

Table 5 Effect of functions on nucleotide replacement lengths in the amniote ancestor-to-contemporary β lineages

Type of residue positions	No. of positions	Average length	Nucleotide replacements per position per 100 Myr
Haem contacts	21	0.43	0.02
Non-haem contact $\alpha_1\beta_2$ contacts	10	0.40	0.02
Salt bridges, associated Bohr effect, $\beta\beta$ contact	4	0.00	0.00
2,3-DPG binding	4	1.75	0.10
Non-salt bridge $\alpha_1\beta_1$ contacts	16	2.81	0.16
Remaining interior positions	21	1.57	0.09
Remaining exterior positions	70	3.34	0.20
All positions	146	2.27	0.13

Positions are grouped according to their functional roles, as may be identified in Table 1; unaugmented nucleotide replacements over the amniote (chicken-mammal) β region of the globin genealogy are averaged for the positions in each group. The times of the connecting links over the amniote β region, when added up, represent about 1.8×10^9 yr.

of helical segments⁴⁸. An alanine to proline mutation at most positions would be detrimental but at certain positions would be advantageous by producing a more stably directed helix. The greater incidence of proline at such positions in human haemoglobin than in ancestral primitive haemoglobins may be attributed to positive selection bringing about further improvements.

Continuing Darwinian evolution

The positions in amniote haemoglobins which have undergone the most evolutionary change are those surface or exterior positions without sharply defined functions (Tables 4 and 5). This apparently supports the neutralist argument that most amino acid substitutions can be accounted for by mutations which escape the scrutiny of natural selection⁸². These surface changes, however, can also be interpreted on selectionist grounds. Having fixed the features in the haemoglobin tetramer responsible for its crucial (and more obvious) functions, natural selection would then be able to shape the finer adaptations of the protein. These adaptations should especially involve the exterior sites, because of their potential for interacting with other macromolecules. Amino acid changes in these exterior positions can be viewed as further steps in an optimisation process in which the functions of proteins become more effectively coordinated. Evolutionary change at the molecular level must correspond in some way to the genotypes of whole organisms. Since the net balance of many gene actions guides organismal selection⁸³, the finer coadaptations among proteins must await the coarser adaptations. While these are being selected a proportion of amino acid substitutions, at the less critical sites, should indeed fit the neutral mutation model. Positive organismal selection, however, eventually improves the subtler protein interactions. Positive and then stabilising selection might well explain the rapid rate of haemoglobin evolution in the early placental mammals and the later greatly diminished rate in hominoids.

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letters to nature

Cosmic rays and the Galaxy

EVIDENCE on whether the majority of detected cosmic rays originate in our Galaxy has been inconclusive: arguments based on the abundance of uranium nuclei, the power available in supernovae, γ -ray emission, the absence of a 10^{20} eV cutoff, and the galactic origin of the electron component all involve uncertainties; but in each case a galactic origin seems to offer a more probable interpretation than an extragalactic origin. Near isotropy has until now been the discordant feature. Work by Krasilnikov *et al.*¹ and Bell *et al.*² now suggests that the arrival directions of the most energetic cosmic rays are almost certainly highly anisotropic. Although the distribution of arrival directions is quite different from that predicted by Karakula *et al.*³ for particles from galactic sources, we point out here that the evidence now indicates strongly a galactic origin for even the most energetic cosmic rays that reach us; we also suggest that there is a very extensive ordered magnetic field outside the plane of our Galaxy.

A 'local' (that is, galactic) magnetic field which is incapable of completely trapping locally produced particles cannot introduce anisotropy into radiation that originated in very distant parts of the Universe, and is therefore supposedly isotropic; so it is necessary to seek a localised source essentially in the Virgo Supercluster or in our Galaxy. Figure 1 shows the distribution of arrival directions of 84 showers above 2×10^{19} eV (from ref. 4). They are plotted in celestial coordinates (Fig. 1a) and the distribution in galactic longitude of showers north or south of the galactic plane is also shown (Fig. 1b). Another 20 showers of almost this energy are included (Fig. 1a) as small points (D. D. Krasilnikov, unpublished); they reinforce the statistical significance of the anisotropy¹, but they are ignored in (Fig. 1b), as without them the data represent an almost uniform exposure to all parts of the sky.

The RA distributions in the north and south hemispheres have significant first and second harmonics respectively, of amplitude $>60\%$, associated with clustering at L, M, N (see Fig. 1a). The existence of widely-spaced maxima, and the wide spread of the most energetic particles do not suggest propagation from a single distant source such as the cluster centre in Virgo. If the supercluster is equivalent to 3,000–10,000 galaxies like our own, but at 18 Mpc as against 9 kpc, the inverse square law alone gives our Galaxy an advantage of more than 400 even if it does not trap cosmic rays; that is, unless it is a weaker than average source. At GeV energies it is not, as synchrotron radiation from our Galaxy and from the possible weak belt of emission from the supercluster⁵ are in about this ratio; and the strong sources Virgo A and Centaurus A (see Fig. 1a) do not change the balance significantly¹². Moreover, the cosmic-ray energy spectrum to the highest energies⁶ now seems essentially continuous with that at GeV energies, in spite of earlier estimates⁷.

Three aspects of the observed anisotropies accord well with a galactic origin, however, when observations at all energies are considered. First, the time of maximum intensity, which in the northern hemisphere switches from ~ 19 h sidereal time below 4×10^{15} eV ($=E_0$), to ~ 13 h above this energy⁸, is still near 13 h at 5×10^{18} – 10^{20} eV (ref. 1). This suggests that one mode of propagation below E_0 , consistent with an escape along magnetic field lines, is overtaken above E_0 by a more rapid drift, perhaps radially outwards across field lines, bringing maxima more normal to

the galactic plane⁹. This motion could persist to the highest energies.

Second, above E_0 , the cosmic-ray flux does indeed fall more steeply, and if it is supposed that the galactic sources inject particles at the rate $Q(E)dE \propto E^{-2.5}dE$ (say), one has an intensity modified by the falling lifetime, $T(E)$, of retention in the Galaxy: $J(E) \propto Q(E)T(E)$. Thus, if the anisotropy, A , varies inversely as trapping lifetime, as should be approximately true, $A(E) \propto Q(E)/J(E)$. In Figure 2, the line $E^{-2.5}/J(E)$ (from ref. 6) is superimposed on a plot of anisotropy measurements, with selected data of high statistical weight^{1,2,10}, an average of many results near 10^{17} eV, and harmonic amplitudes present in other tables^{7,11}. The energy dependence of anisotropy, and the form of the energy spectrum, can be seen together to suggest an explanation in terms of more rapid escape from the Galaxy above E_0 .

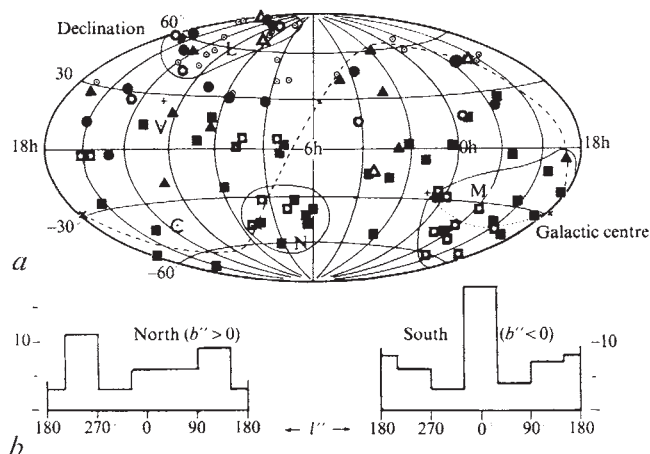


Fig. 1 a, Arrival directions of 84 showers above 2×10^{19} eV. ■, Sydney; ▲, Volcano Ranch; ●, Haverah Park. The most energetic 30%, are shown as open symbols. Twenty showers above 10^{19} eV: ○, Yakutsk. Plotted in coordinates of right ascension (increasing to left, with 6 h in centre) and declination. The galactic plane is shown dashed, galactic poles as crosses. The dotted line joins the south galactic pole to the galactic centre. b, Distribution of the 84 showers in galactic longitude, plotted separately for north and south galactic latitudes. V, Virgo A; C, Centaurus A; L, M, N, clustering.

Third, returning to the highest energies, the maximum M, the most prominent in the harmonic analysis, which is responsible for the peak in Fig. 1b (south), is centred on galactic longitude 0° (Fig. 1a). It lies south of the region of strong synchrotron and gamma-ray emission, as though deflected by a large-scale magnetic field directed towards galactic longitude 270° to the south of the plane, between us and the centre of the Galaxy. The group N (Fig. 1a) may be accidental, but lies roughly in the Vela direction, along the galactic field. The unexpected³ absence of any concentration in the 90° direction, may mean that of the few sources effective at any one time in this energy range, none lies in that direction. But why peak L is displaced in galactic longitude is not clear.

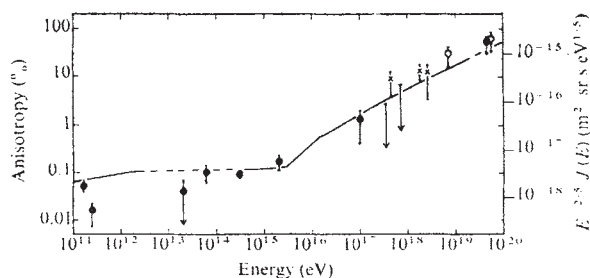


Fig. 2 Anisotropy in the right ascension distribution of cosmic rays of different energies, E , along a broad belt of constant declination. The larger of the first (●) or second (○) harmonic is plotted. ×, Unpublished harmonics present in tables of shower distributions, but only showers in one hemisphere (positive or negative declination) are included. The line is the inverse of the cosmic-ray energy spectrum, multiplied by $E^{-2.5}$.

To retain nuclei of $\sim 10^{20}$ eV would require an ordered magnetic field extending several kpc from the galactic plane; a 2 μ G field could retain oxygen nuclei, but not lighter nuclei. There is independent evidence, from the extension of observed synchrotron radiation to 1 kpc, that the scale of decay of the magnetic fields must exceed 1 kpc.

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Measured offset between the Crab pulsar and Tau X-1

WE have used the MSSL X-ray detector on board the Copernicus spacecraft to measure the effective diameter of the Crab Nebula and to measure the location of the centroid of the emission with respect to the accurately known pulsar position¹.

On October 7, 1974, the orbit of the satellite took it through the Tau X-1 lunar occultation 'shadow' on two successive orbits, enabling us to observe two disappearances (D_1 and D_2) and two reappearances (R_1 and R_2) of the Crab Nebula with a collimated proportional counter operating in the energy range 2.5-7.5 keV. All four occultations occurred partially during the dead time of the spacecraft data handling system (23.6 s out of every 86.5 s), though R_2 started so close to the end of a live period as to make the data statistically less reliable than the data from the other events.

Figures 1 and 2 show the counts from the detector in successive 3.3-s time intervals during the occultations. No background has been subtracted; the low level of background which is apparent when the source is occulted does not vary by more than 10% during each event, as monitored by the guard counter associated with this detector².

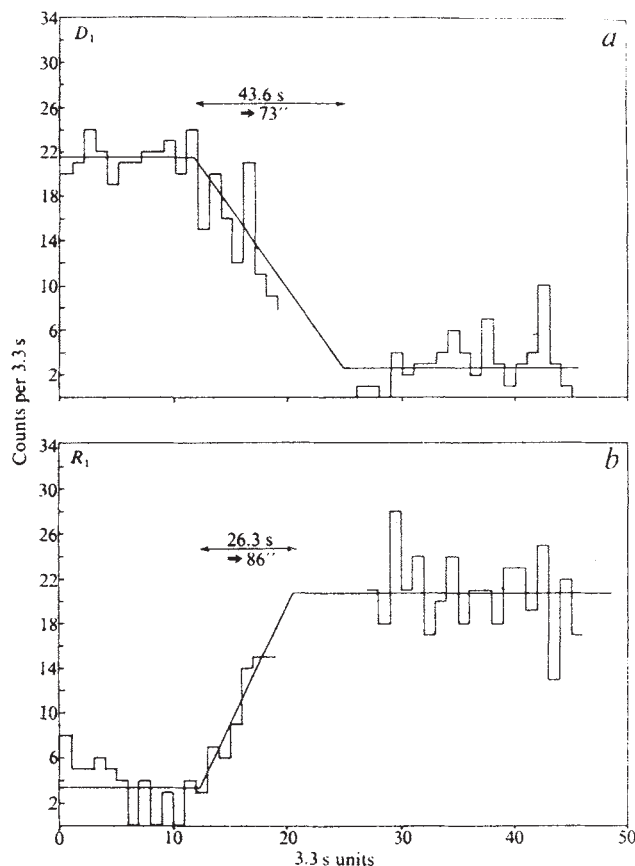


Fig. 1 2.5-7.5 keV count rate during first disappearance (a) and reappearance (b). Background has not been subtracted. The solid lines show the best fit to the data. Orbit 11244, $p. a = 236^\circ$.

The background level does vary from occultation to occultation because of the changing geographical location of the satellite. Figures 1 and 2 also show the best fitting constant count rate before and after each occultation and a linear change in count rate during the course of the occultation. The data do not justify a more complex description. The best fit is defined as that combination of the time of change of slope (t_0) and the value of slope (S) which gives a minimum value of χ^2 when compared with the data. Values of χ^2 were computed for a range of values of t_0 and S and the resultant χ^2 grid was examined to find the minimum. The uncertainty in centroid location was derived by finding the uncertainty in t_0 when S was held fixed at its most probable value; the one sigma uncertainty in source diameter was derived from the maximum and minimum values of S reached by the $(\chi^2_{\min} + 1)$ contour. It is normally recommended³ that interval widths in χ^2 tests should be wide enough to include a minimum of five events each. We have therefore summed the count rate in 3.3-s bins, rather than use our maximum time resolution of 1.65-s, in order to compute valid estimates of χ^2 for each trial fit. Such a summation still leaves R_2 with only two intervals containing more than five events, and so we cannot obtain valid χ^2 values for these data. We therefore restrict discussion to the other three occultations.

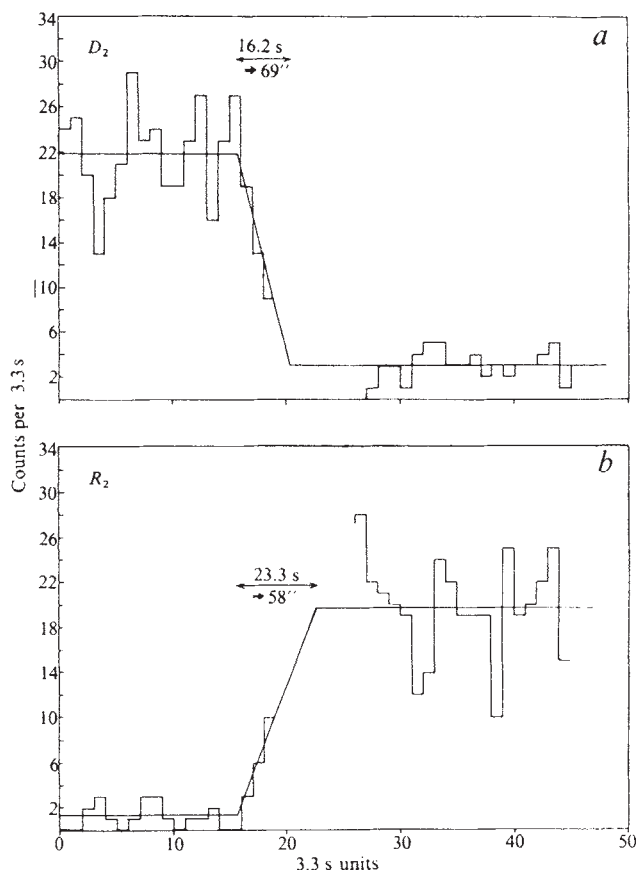


Fig. 2 2.5–7.5 keV count rate during second disappearance (a) and reappearance (b). Background has not been subtracted. The solid lines show the best fit to the data. Orbit 11245, p. a. = 273°.

We used the best estimates of S and t_b to define the effective times at which the occultations started and finished. The apparent position of the Moon's limb relative to the pulsar at these times was calculated using satellite positions derived by the Goddard Space Flight Center tracking group and the lunar ephemeris currently in use at the Royal Greenwich Observatory. We have neglected the irregularities and curvature of the lunar limb.

Table 1 gives the measured effective diameters together with the uncertainties in diameter and in the location of the centroid (measured along the line perpendicular to the lunar limb). A weighted average for R_1 and R_2 is given, as the position angles for these events are within 5° of one another. Figure 3 shows these diameters and centroid locations relative to the pulsar position.

Most of the X-ray flux is asymmetrically disposed with respect to the pulsar. A similar result is suggested by W. Lewin (private communication) from a recent balloon flight⁴. Our effective diameter of 73'' at position angle (p.a.) 236° is similar to the balloon measurement (60'' ± 10'') at p.a. 244°, though our value of 71'' at p.a. 275° is substantially larger than the value of Ricker *et al.*⁴ of 30 ± 10'' at p.a. 130° (≡ 310°). The fact that the angle between our occultation passes was less than that in the balloon measurements (39° compared with 66°) would produce some sort of averaging, but not sufficient to account for the whole difference.

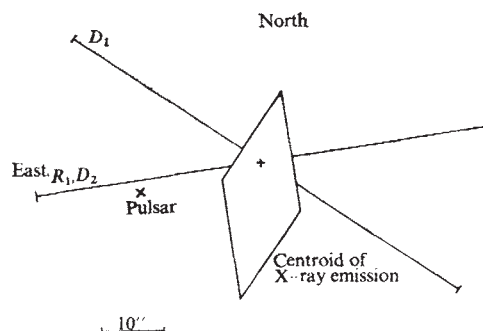
With the exception of the 1964 occultation experiment of Bowyer *et al.*⁵, all previous estimates for the X-ray diameter of the Crab Nebula have measured some form of effective diameter, using several different techniques. Our estimates of 73'' and 71'' are in reasonable agreement with the figure of 100'' from a modulation collimator experiment⁶, 66''

Table 1 Measured effective diameters

Event	Diameter	1 σ Error	Uncertainty in centroid location (1 σ)	Position angle
D_1	73''	+38'' -38''	+8'' -4''	236°
R_1, D_2	71''	+15'' -8''	+4'' -7''	275°

from a 25–100 keV modulation collimator experiment⁷ and 83'' from a raster scan with the arc minute angular response detectors on the Copernicus satellite⁸. As was clear from these earlier experiments, the X-ray emitting region is significantly smaller than the optical nebula, though not as small as would be expected if models such as that of Wilson⁹ were fully representative of the situation. There also seems to be a lack of any dependence of the X-ray size on photon energy, as may be judged from the basic similarity of the balloon energy results^{4,7} to the lower energy rocket or satellite results. Although Wilson's model could be forced to predict a size comparable to that observed by allowing the relativistic electrons to originate in an injection region of diameter 20'' rather than in a point source, the model still requires a decrease of angular diameter by some 45% in going from 3 to 30 keV effective measurement energy. This is clearly at variance with the observations. An alternative solution suggested by Wilson was the possibility that the electrons which radiate at X-ray energies have a larger diffusion coefficient. The particular model described was the "magnetic cloud" model (ref. 10, p. 170) which leads to a predicted 75% increase in diameter in going from 3 to 30 keV. This too is at variance with the observations. It is possible that the electron injection region could still be of significant angular diameter, and that the energy dependence of size of the X-ray emitting region could somehow be suppressed, but in view of the significant displacement between the emission centroid and the pulsar, which is generally presumed to be the ultimate source of the electrons, one must search for some reason why these electrons should generate X rays predominantly in the region to the west or north-west of the pulsar. Rees and Gunn have suggested¹¹ that the electrons are carried outwards by an expanding magnetic field. If this is so, the observed offset between the pulsar and the X-ray emission region requires either the magnetic field to be expanding from a point not coincident with the pulsar (a second electron injection source in addition to the pulsar?), or the presence of substantial amounts of nebular matter to the south-east of the pulsar which

Fig. 3 Our measured diameter along position angle 236° (D_1) and 275° (average of D_2 and R_1). The diamond shape is the area common to the 1 σ error bands of the centre of each 'diameter'. The offset from the pulsar position is clearly seen.



would prevent expansion in that direction. In this context, it may be significant that photographs of the filamentary structure of the Crab¹² show a toroidal filament about 1' in diameter just to the north-west of the pulsar, whilst the peak of the optical continuum¹² is located at approximately the same position angle and distance from the pulsar as is our measured centroid.

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Ultraviolet emission lines in the spectrum of Procyon

THE Princeton instrumentation¹ on the satellite Copernicus has been used to observe the F5 IV star, α CMi (Procyon). In addition to previously observed² lines of Mg II, the Lyman- α line of H I (1,216 Å), and the resonance lines of Si III (1,206 Å) and O VI (1,032 Å) have been observed for the first time in an F-type star. Figure 1 shows the observed lines, all of which exceed the two standard deviation level.

The observed Lyman- α profile clearly contains a component due to absorption by interstellar atomic hydrogen in the line of sight to Procyon. It is possible to calculate the range of interstellar hydrogen column densities N_H that would lead to the observed profile, by using the equation for the optical depth in the damping wing of Lyman α , that is $\tau(\lambda) = 9.25 \times 10^{-20} (\lambda - \lambda_0)^{-2} N_H$, where λ_0 is the wavelength of the line centre in Angstroms. Taking the attenuation at each point in the line to be $\exp(-\tau(\lambda))$ gives

$$1.5 \times 10^{17} \text{ cm}^{-2} \lesssim N_H \lesssim 3.0 \times 10^{17} \text{ cm}^{-2}$$

Since the distance³ to Procyon is 3.5 pc the mean density of atomic hydrogen, n_H , lies in the range $0.015 \text{ cm}^{-3} \lesssim n_H < 0.030 \text{ cm}^{-3}$, which is much lower than the value of 0.3 cm^{-3} deduced^{4,5} from observations of early type stars at distances of ~ 100 pc. A similar low value of $n_H = 0.015 \text{ cm}^{-3}$ has been calculated⁶ from the Lyman- α absorption profile in the K star α Boo (Arcturus) which is 11 pc away. So it seems that the neutral hydrogen density in the solar neighbourhood (say to ~ 10 pc) is much lower than the average value out to ~ 100 pc. The low value derived implies that the opacity of the nearby interstellar medium at $\lambda < 912$ Å (the H I Lyman limit) is considerably less than previously assumed and even at 10 pc there should be

about 10% transmission at the Lyman edge and greater than 50% transmission below the He I limit (504 Å). The possibility of making observations in this spectral region in nearby stars is thus limited solely by the intrinsic stellar emission and not by interstellar absorption.

The observed emission line fluxes have been converted to absolute energy fluxes at Procyon using the known angular diameter of the star⁷. Using methods established for analysing the solar extreme ultraviolet spectrum⁸ these fluxes can be converted to the emission measure, $\int n_e n_p dh$, for the temperature region where each line is formed. Figure 2 shows the emission measures observed and the mean solar distribution⁹. A range of possible values is indicated for Si III since the

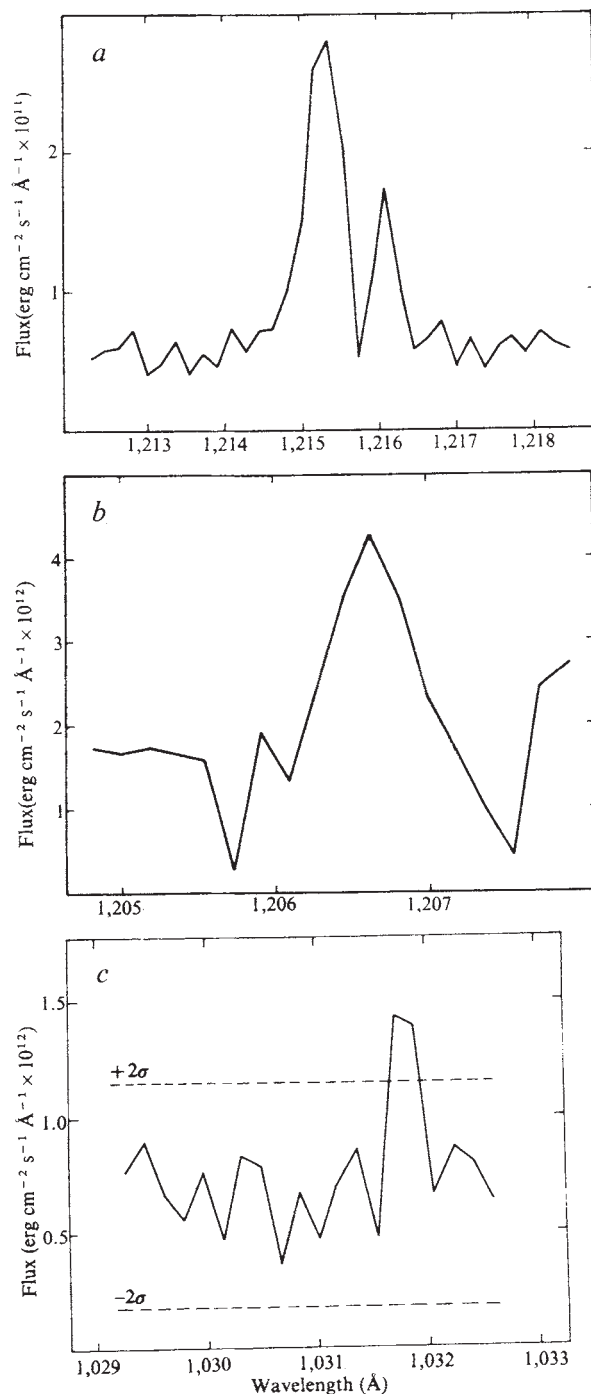


Fig. 1 The observed emission lines of: a, H Lyman- α at 1,216 Å; b, Si III at 1,206 Å; c, O VI at 1,032 Å.

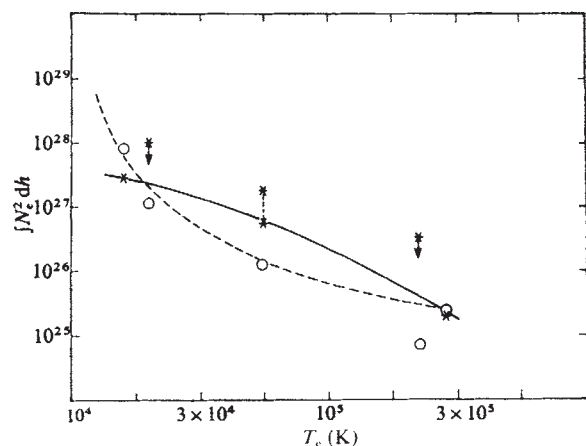


Fig. 2 The emission measures, including upper limits, derived from the observed fluxes from Procyon. The corresponding solar values and the mean solar values are also shown. $\circ$, Sun; $\star$, Procyon.

ground state population is uncertain because of the existence of a metastable level. Interpolating between the observed values gives an emission measure distribution as a function of temperature. This can be used to derive the range of models which would satisfy the emission measures¹⁰ and also fit on to the model of the low chromosphere of Procyon made by Ayres *et al.*¹¹. These models depend on the boundary value of the electron pressure at $T_e = 3 \times 10^5$ K (where O VI is formed) which cannot be determined directly from the present observations. But an upper limit of $N_e T_e = 1.7 \times 10^{14} \text{ cm}^{-3} \text{ K}$ is established by assuming the same total pressure as derived by Ayres *et al.*¹¹, at the lower temperature of 8×10^3 K and a lower limit of $N_e T_e = 2.0 \times 10^{13} \text{ cm}^{-3} \text{ K}$, is obtained by assuming that O VI is formed in an isothermal atmosphere at $T_e = 3 \times 10^5$ K. The chromosphere—or transition region—of Procyon is therefore less dense than the corresponding parts of the solar atmosphere but must be thicker in order to match the emission measures.

The models derived for the two limiting cases have been examined for energy balance. The high pressure model shows the existence of a true transition zone, a region where conductive energy flow is dominant, indicating the presence of a corona at greater heights. But the onset of the transition region occurs at a significantly higher temperature (10^5 K) than in the case of the Sun (3×10^4 K). The low pressure model shows no such transition zone, the conductive flux being small throughout the observed temperature range. Radiative losses must therefore be balanced by the direct dissipation of the primary mechanical energy flux, probably shock heating by acoustic waves, and in this sense the model is better regarded as an extended chromosphere.

So the chromosphere of Procyon extends to higher temperatures than in the Sun; but the detection of a true corona will need additional investigations. A lower limit to the maximum temperature of any corona is given by the temperature of the highest excited line observed (O VI) $T_{\text{max}} \lesssim 3 \times 10^5$ K. An upper limit cannot be derived directly from the observations but overall energy considerations give $T_{\text{max}} \lesssim 6 \times 10^6$ K.

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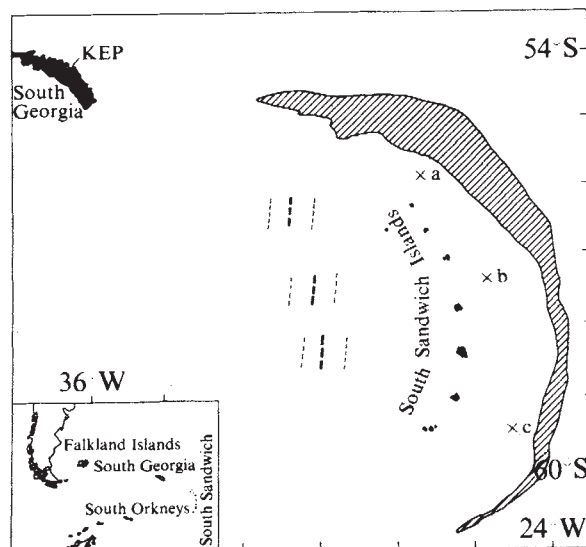
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Seismic wave attenuation and velocity anomalies in the eastern Scotia Sea

As part of a more general investigation of the structure and plate tectonics of the Scotia Sea early in 1972 a temporary seismographic station was installed at King Edward Point, the British Antarctic Survey station on South Georgia (see Fig. 1). The station (KEP) comprises a three-component set of short-period Willmore seismometers at position 54.28° S , 36.48° W , and two single channel (vertical) outstations with telemeter links to KEP; all five channels record on magnetic tape. Interest has been concentrated on a study of activity associated with the South Sandwich Islands subduction zone¹.

Figure 2 shows typical vertical component seismograms observed at KEP for three earthquakes in the South Sandwich Islands. Event (a) is located in the north, event (b) in the centre and event (c) towards the southern end of the arc (see Fig. 1). On replay a bandpass filter of 0.5–10.0 Hz was used for all

Fig. 1 The East Scotia Sea. The hatched area represents the South Sandwich Trench at depths greater than 6,000 m. The spreading centre, identified by magnetic anomalies, is shown as thick dashed lines with the 1 Myr isochron shown as a thin dashed line. From bathymetric data the spreading centre is assumed to extend to the trench in the north, and southwards to the South Scotia Ridge. a, b and c, Locations of the three earthquakes discussed in the text.



records. Event (a) is at a range of 5.54° and the seismogram shows distinct P and S phases. On the seismogram of event (b), at a range of 6.78° , there is no clear onset of the S-wave phase but some emergent, low frequency energy can be seen around the expected S-wave arrival time. There is no sign of any S-wave energy on the record of event (c) at a range of 8.09° . These features are also observed on both of the horizontal component records of each event.

Figure 3 shows the P-wave arrivals from each of the three events in detail. The attenuation of the higher frequency P-wave energy from the central and southern events is quite striking: the dominant frequency is reduced from approximately 5 Hz for event (a) to approximately 1 Hz for event (c).

For relocation purposes the arc was divided into three regions: $55\text{--}57^\circ$ S, $57\text{--}59^\circ$ S and $59\text{--}61^\circ$ S. For each of these regions, approximately 20 large, well recorded events (post 1964) were selected and relocated using a Joint Epicentre Determination program². This reduces source bias and determines a set of station corrections for each of the three regions³. Subsequently, events were relocated using a single event program, with the appropriate station corrections applied. This procedure has been applied to all events which occurred after 1964 and it

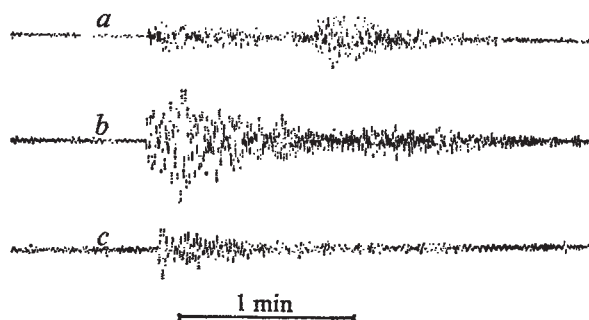


Fig. 2 Typical vertical component wave forms observed at South Georgia for the three events shown in Fig. 1. Record a is at quarter gain.

results in a marked improvement in clustering of the hypocentres. Since we are here concerned with local travel-time anomalies we have not used the South Georgia data in the relocation procedure. Phase data have been taken from the United States Coast and Geodetic Survey Earthquake Data Report lists, and also from additional station bulletins.

The travel-time residuals at KEP, relative to Jeffreys-Bullen times, of 36 recent, relocated events have been plotted against epicentral latitude (Fig. 4). The northern group of 25 events show negative residuals, with a mean value of -4.0 ± 1.4 s. The nine southern events, on the other hand, have large positive residuals with a mean value of $+6.3 \pm 1.1$ s. The residual scatter within the groups is probably a result of the fact that only distant stations have been used for location (South Pole, SPA, at an average range of 3,700 km is often the nearest station), giving poor discrimination between depth and origin time. So far, the number of events recorded from the central region of the arc has not been sufficient to allow accurate mapping of the transition from negative to positive residuals;

Fig. 3 Detail of P-wave arrival for the events shown in Fig. 2. Record a at quarter gain.

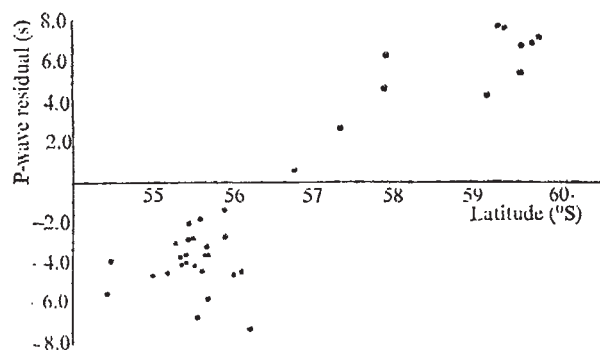
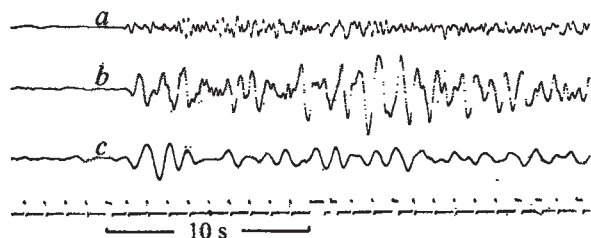


Fig. 4 P-wave travel time residuals observed at KEP plotted against epicentral latitude.

however, the few available results suggest that the transition is abrupt. The variation in the residuals over the length of the arc is considerable and indicates a mean velocity difference of approximately 10% between the northern and southern ray paths.

Karig⁴ has suggested that the basins behind the western Pacific arcs are of extensional origin, and have been formed by diffuse upwelling of hot mantle material. This has been supported by the discovery of a low Q , low-velocity zone behind the Tonga Island Arc^{5,6} where an upper mantle velocity of 7.1 km s^{-1} has been observed beneath the Lau Basin. This compares with an upper mantle velocity of 8.45 km s^{-1} beneath the old Pacific Basin to the east of the Tonga Arc⁶. In the region under study here, Barker⁷ has identified an active spreading centre, orientated approximately north-south along longitude 30° W (see Fig. 1), with a spreading rate of $70\text{--}90 \text{ mm yr}^{-1}$. For three-quarters of its path the energy from southern events crosses an oceanic area which is less than 8 Myr old, whereas the ray paths from the northern end of the arc pass through old, normal, oceanic lithosphere. The anomalous velocities and attenuation observed here are, therefore, very similar to those which occur in the marginal basins of the western Pacific and are consistent with the presence of a high temperature zone of mantle at shallow depth. The similarity is interesting in view of the fact that behind the South Sandwich Arc the basin shows all the usual features of mid-oceanic ridge spreading.

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Manganese ore deposits and plate tectonics

It is now generally accepted that the oceanic crust and overlying pelagic sediments are enriched in several metals, including copper and manganese¹⁻³. The theory of plate tectonics has demonstrated that the oceanic lithosphere is likely to be remobilised or emplaced along convergent continental margins through magmatic or tectonic activity. The plate tectonic model has been used to explain the metallogenesis of copper⁴⁻⁸, and these concepts have been extended to manganese mineralisation along orogenic belts^{9,10}. We demonstrate that plate

consumption along subduction zones gives rise to at least three processes which may be associated with production of manganese deposits.

One type of manganese mineralisation is related to calc-alkaline magmatism. Reports on porphyry copper deposits and numerous manganese ore deposits have revealed that the two mineralisations seem to be contemporaneous within calc-alkaline terrains¹¹. Porphyry copper deposits consistently occur over active and recently active subduction zones throughout the circum-Pacific, Caribbean, and Alpine orogenic belts, except in Japan where stratabound copper sulphide deposits predominate¹². Manganese mineralisation is also found in these Mesozoic and Cainozoic porphyry copper deposits (including Japan), as well as older terrains (Palaeozoic and perhaps Precambrian) from which porphyry copper deposits have presumably been eroded. Porphyry copper deposits are usually found in calc-alkaline stocks and cupolas underlying strato-volcanoes, although copper mineralisation is a rather late event in the emplacement of the entire calc-alkaline suite⁴⁻⁸. Similarly, many manganese deposits are also closely associated with calc-alkaline igneous rocks, particularly andesitic tuffs and breccias, usually in the vicinity of volcanic centres. Furthermore, these manganese deposits seem to be formed after the main phase of volcanic activity¹³. Sillitoe⁷ has proposed a plate tectonic model for the origin of porphyry copper deposits, which embodies the concepts of seafloor spreading, transform faulting, underthrusting at continental margins and island arcs, and the generation of calc-alkaline magmas by partial melting of oceanic lithosphere. He has also suggested⁸ that typical porphyry copper deposits occur in the upper levels (at depths of 1.5–3 km beneath the summits of stratovolcanoes) of porphyry copper systems which may possess vertical extensions as great as 8 km.

The close space-time relationship between manganese deposits, porphyry copper deposits, and subduction zones indicate that both manganese and copper are incorporated into melts which rise to form calc-alkaline volcanic chains and co-magmatic roots⁹⁻¹¹. These magmas are generated on or above Benioff zones by partial fusion of oceanic crust rich in metals and pelagic sediments, and incorporate volatiles derived from subducted oceanic lithospheric plates¹⁴⁻¹⁸. After the metals become incorporated into the magmas forming the roots of volcanic chains, solutions from which ores may form are expelled from cupolas of these roots, leading to brecciation and intense hydrothermal activity characteristic of porphyry copper systems. The metals are probably transported in hydrothermal fluids and solutions as complex Mn(II) and Cu(II) ions. At lower temperatures in aqueous environments, hydrolysis occurs leading to the precipitation of extremely insoluble sulphide, oxide and metallic phases of Cu. Manganese is transported to higher levels in the porphyry copper system and may escape out of the volcanic pile into the submarine environment where it is deposited as Mn(II) carbonates and silicates, or where oxidation to very insoluble Mn(IV) oxide minerals takes place.

A second location of manganese ore deposits is in ophiolites, which comprise, in ascending order starting from the stratigraphic bottom, ultramafic bodies, complex gabbro intrusives basalt pillow flows and sediments. This sediment layer, although metamorphosed to varying degrees, frequently contains substantial manganese deposits. Examples of such mineralisation are found throughout the western coast of North America and throughout the western circum-Pacific, particularly in Japan. The Alpine belt is also well endowed with manganese deposits in ophiolites, particularly in Morocco. Other examples include Cuba, the Urals, and eastern Australia. The ages range from the Precambrian to the Cainozoic. The remarkable resemblance of ophiolites to oceanic floor, both in terms of chemistry and geometry, has prompted the widespread belief that ophiolites are indeed oceanic floor, emplaced tectonically into orogenic belts^{19,20}. Ophiolite emplacement is integrally associated with plate subduction, either by the accretion of

oceanic crust on to the wall of the upper plate (in oceanic trenches) or by the bodily thrusting (obduction) of the oceanic crust on to a continental margin¹⁹⁻²². The principal difference between the two mechanisms is the direction of subduction relative to the continental margin. Both cases are associated with blueschist (glauco-phane) metamorphism and melange terrains.

The widespread occurrence of manganese in the uppermost layers of ophiolites is understandable when one considers the abundance of manganese in the sedimentary layers of the oceanic crust. Deep sea drilling projects^{23,24} and geochemical studies across oceanic rises²⁵⁻²⁷ have revealed a sediment layer rich in manganese oxide apparently generated by the volcanism occurring at the rises. Oceanographic cruises have mapped extensive areas of ferromanganese nodule deposition on the sea floor, particularly in the north-east Pacific Equatorial Region²⁸⁻³⁰. These sediments, or their metamorphosed equivalents, are frequently, if not typically, associated with ophiolite terrains.

A third location of extensive manganese mineralisation exists within the small marine basins located behind island arcs. Examples include the Philippine Sea and the Sea of Japan. One occurrence of a manganese deposit in an existing marginal basin is the Mariana Trough near Guam in the western Pacific³¹. This was a 6 cm wide vein of manganese oxide containing 48.0 weight % Mn. But a larger scale example is the Atasu region in central Kazakhstan, USSR, which we interpret as an uplifted Palaeozoic marginal basin.

Marginal basins are thought to be produced by thermal diapirs rising off subduction zones at depths of 200 to 400 km (ref. 32). These thermal diapirs are essentially rising zones of high heat flow generated at the surfaces of subducted lithospheric slabs. As a diapir rises upward, it upwells and spreads outward so as to force the basin floor apart. The high heat flow near the surface of the basin triggers volcanism which fills the widening rift with basaltic magmas, thereby producing new oceanic crust. Marginal basins are therefore bounded by steep scarps marking the original walls of the initial rift valley^{21,33}. As spreading commences and sediments rapidly fill the developing trough, continued normal faulting pervades both the new basaltic crust and the overlying sediments. Except for the volcanogenic andesitic flysch apron at the arc side, the marginal basin represents the typical miogeosynclinal environment of manganese deposition. By analogy with the processes occurring at oceanic rises, the median rifts of marginal basins are expected to be the sites of manganese emanations, although the median rifts will be overlain by a thick and highly faulted sequence of sedimentary materials. Solutions rich in manganese reach the surface through the fault conduits. Indeed, the manganese mineralisation in central Kazakhstan is intimately related to the normal faulting which characterises the complex Kazakhstan graben³⁴. Continuous sedimentation accompanied the spreading of the central Kazakhstan trough so that the oldest sediments are now found buried at the flanks of the Palaeozoic basin. As seafloor spreading continued, sedimentation progressively buried newly formed crust and became the host of manganese mineralisation.

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Magnetic susceptibility stratigraphy of Pacific Pleistocene sediments

FROM studies on a few gravity cores¹ it is known that the absolute susceptibility of Pacific sediments ranges from 10^{-5} to 10^{-6} e.m.u. g^{-1} oersted⁻¹. Using a simple, non-destructive and rapid logging technique², we measured the relative susceptibility, as a function of depth, in 86 gravity cores from the Pacific (Core Library, Scripps Institution of Oceanography). We report the salient features of this investigation (details will be presented elsewhere).

Figure 1 shows the location of the cores and Fig. 2 the three types of susceptibility logs obtained. Type 1 occurs mostly in the trench and sediment trap areas, each peak in the magnetic susceptibility (Fig. 2) probably representing an episode involving the deposition or slump rich in volcanic material. Such events, which occurred in

Fig. 1 Location of cores in the Pacific. Dashed double lines, principal trench systems. ●, Type 1 logs; ×, type 2; ■, type 3.

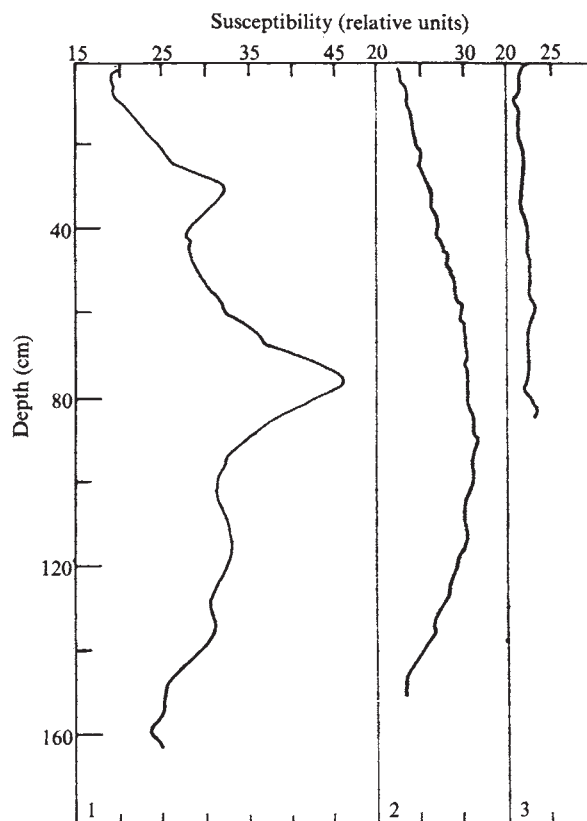
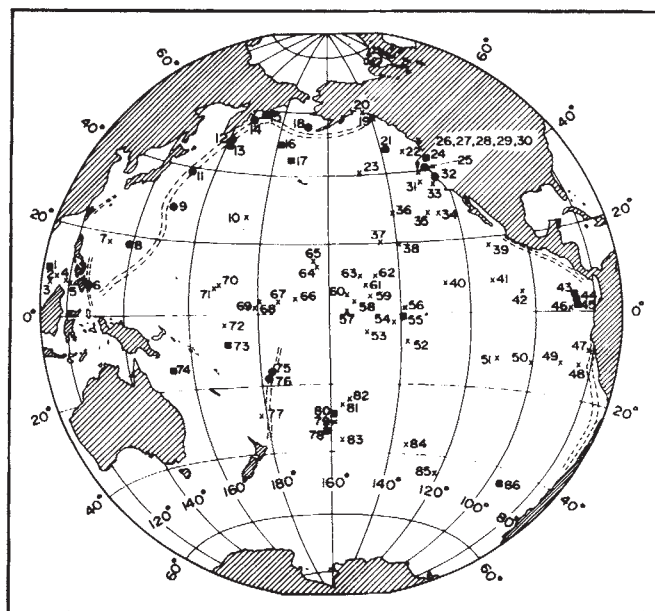


Fig. 2 Typical examples of the three types of log. Number underneath each curve indicates the type.

the period 0.2–2.8 Myr ago, were sometimes synchronous in large areas representative of type 1 and sometimes localised.

Type 2 are the most common; 66% of the cores studied belong to this type and have a single, broad hump in the susceptibility log (Fig. 2), the maximum of which occurs anywhere between 40 cm and 120 cm. These events occurred in the period 0.2–2.5 Myr ago.

Four explanations are possible, one of which is the oceanwide deposition of volcanic material. Regions containing sediments of type 1, by virtue of their proximity to the volcanic activity, register a sharp event and the sediments from the rest of the Pacific basin register only a diluted or smeared event which may appear as a broad hump covering the same time span. An increase in the amount of atmospherically transported magnetic material (cosmic, volcanic and terrestrial)³ deposited on the Earth in the past, could be responsible, an observation supported by studies on slow-growing manganese nodules^{4,5} and meteorites⁶. A decrease in the accumulation rate of sediments, which would in effect manifest itself as an increase in the magnetic material depositing at that time, is another alternative. The hump could also result from slow chemical or physical changes after deposition, which may affect, for example, the composition or state of seawater itself over the time period represented by the hump. To ascertain the relative importance of these explanations, detailed work involving mineralogy and geochronology of the cores of type 2 is necessary.

About 15% of the cores belong to type 3, in which the susceptibility is approximately constant with depth (Fig. 2). A few of these cores also show a single, broad hump, as for type 2 but the hump is very flat and is distinguishable from the former.

The accumulation rates of the Pacific sediments had to be ascertained from the literature as none of these cores was dated. Some of the cores, especially of types 1 and 2 could be dated using beryllium-10 (ref. 7) and ionium/thorium techniques⁸. The maximum of the broad hump in type 2 seems to be a fairly well distributed event in the whole Pacific and may act as a stratigraphic marker. To detect this marker in a gravity core (about 150 cm) takes no more than half an hour. As has already been stressed, such a log will also indicate the suitability of a core for palaeomagnetic reversal studies²; types 2 and 3 are ideal. Further investigations of this kind on piston and Deep Sea Drilling Project cores, together with the mineralogy and geochronology, may prove very useful for establishing the magnetic susceptibility stratigraphy of the world ocean sediments.

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Late Pleistocene tropical aridity synchronous in both hemispheres?

THERE is now firm evidence of late Pleistocene aridity in many parts of the tropics in contrast to the belief that high latitude glacial periods were coeval with low latitude pluvial periods¹. A good case may be made for synchronous glacial conditions in both hemispheres during the late Pleistocene^{2,3}, although there are notable and intriguing exceptions⁴. The latter may result from precipitation variations caused by changes in the incidence of local rain-bearing winds, which in turn result from changes in sea level, ocean currents, and, perhaps, from rain-shadow effects. It is reasonable to hypothesise that major climatic changes in extraglacial areas, in particular the non-mountainous parts of the tropics, may also have been synchronous during the late Pleistocene. We propose that the increasing evidence from Africa, India, South America and Australia provides further support for the view that there was widespread tropical aridity in both hemispheres during the late Pleistocene. We will review evidence from the Northern Hemisphere first.

In East Africa, lake levels rose at the start of the Holocene after a long period of low levels and high salinities^{5,6}. Lakes Victoria and Albert in Uganda were low for an unknown time just before 12,500 yr BP (refs 7 and 8), and the late glacial vegetation on Ruwenzori⁹ was significantly less mesophytic

(adapted to a moderately moist environment) than during the Holocene. Lakes in the Kenya Rift Valley, high during the Holocene, were low immediately beforehand^{10,11}. Lake Rudolf on the Kenya–Ethiopia border, fed by the Ethiopian Omo river, was low before 12,000 yr BP and high thereafter⁶. In the southern Afar Rift the lakes were low and saline between about 20,000 and 12,000–14,000 yr BP, rising during the early Holocene^{12,13}. Lake Chad, fed by the Logone and Ubangui-Shari river systems, was very low between 20,000 and 13,000 yr BP, and high from 12,000 to about 5,000 yr BP (ref. 14). Rainfall on to and runoff from the Adamawa Mountains in Cameroon must therefore have been low during the late Pleistocene.

More direct evidence of late glacial tropical African aridity comes from the extensive belt of fixed dunes which now extend down to latitude 10–12° N in Senegal, Mali, Niger, Nigeria, Chad and Sudan^{15–19} over an east–west distance of some 5,000 km. Until the recent drought, seif dunes were mainly active in this region north of the 150-mm isohyet¹⁷. The fixed dunes extend south to about the 800-mm isohyet¹⁷, and calculated palaeo-wind directions suggest a late Pleistocene southward shift of the sand-moving winds in Sudan of about 200–450 km¹⁹. At Adrar Bous in the Ténéré desert of Niger, wind-patinated Aterian tanged points and associated stone tools, firmly dated elsewhere at between about 30,000 and 15,000 yr BP, coincide with a phase of aridity which was followed and preceded by periods of high lake levels in this now dry area^{20,21}. In the valley of the White Nile in central Sudan, linear dunes were active shortly before 12,000 yr BP, when they became partly submerged beneath fluvio-lacustrine clays deposited by the rising White Nile^{22,23}.

There is also abundant evidence of widespread (?) late Quaternary aeolian activity in parts of Angola, Zambia, Mozambique, Rhodesia and Zaïre^{24–26}, although the exact age of these wind-blown sands is still in doubt. The pollen spectra from Angola²⁷ and from Kalambo Falls²⁸ in Zambia, have been better dated, but changes in the ratio of tree to non-tree pollen are related to both rainfall and temperature, and an increase in grassland at the expense of woodland may reflect lower glacial temperatures rather than reduced precipitation. Equally, although the geomorphic evidence for late Pleistocene aridity in west-central Zaïre²⁵ seems valid, we do not yet know whether this aridity is related to slight changes in the cold Benguela Current²⁹, or is in fact more general. A recent reappraisal of published pollen spectra from tropical Africa³⁰ tried to separate inferred temperature changes from inferred changes in humidity. The writers concluded that during the late Pleistocene dry conditions prevailed at Sacred Lake (Mount Kenya), the Cherangani Hills (Kenya), Muchoya swamp (on Ruwenzori) and in the Lake Victoria basin (Uganda).

In the southern Rajasthan desert of north-west India, salt lakes were full of freshwater, and the vegetation was locally mesophytic from at least 10,000 to about 4,000 yr BP (refs 31 and 32). Some of the lakes were formed by the late Pleistocene damming of former river courses by aeolian sands, and wind-blown sands extended as far east as the 850-mm isohyet during the late Pleistocene and at least once beforehand^{33–35}. Late Pleistocene aridity in tropical north-west India therefore seems plausible.

Data from Australia are sparse but informative. In north-west Australia stable seif dunes now extend out on to the continental shelf. Holocene shallow-water marine and estuarine clays unconformably overlie these dune sands, which lack a soil profile and so were probably active until the Holocene³⁶. In deep-sea core V-260 on the Sahul Shelf between Timor and Darwin the rate of clastic sedimentation was rapid during the late Pleistocene and slow thereafter. The carbonate nodules which occur in the Pleistocene but not in the Holocene portions of the core are regarded as pedogenic and as symptomatic of a soil climate drier than the present one^{37,38}. At Lynch's Crater in north-east Queensland a pollen core extending over 60,000 yr indicates a change from a less mesophytic to a more mesophytic regional vegetation from the late Pleistocene to the early Holocene³⁹, and is the most convincing evidence for late

Pleistocene aridity in tropical Australia. Theoretical reconstructions of the late Pleistocene climates of the Torres Strait area in northern Queensland also suggest late glacial aridity¹⁰.

Deep-sea cores from the continental shelf east of Brazil show rapid deposition during the very late Pleistocene of abundant unweathered feldspar and coarse sub-angular quartz sand, changing abruptly to fine grained marine deposition during the Holocene¹¹. Both Holocene marine cores¹¹ and modern sediments carried by the Amazon¹² contain relatively little feldspar, suggesting that chemical weathering was limited and mechanical erosion dominant during the late Pleistocene on the Precambrian Shield areas of Brazil and Guiana, an inference consistent with a climate drier than the present. Pollen studies in lowland tropical South America¹³ likewise reveal a change from late Pleistocene savannah woodland to early Holocene rain forest, much as in Queensland³⁹ and Uganda⁹.

The final line of evidence concerns aeolian detritus in deep-sea cores from the tropical Atlantic. Darwin (in 1946) and Ehrenberg (in 1847) correctly interpreted wind-blown dust in the tropical mid-Atlantic as being of African and probably of Saharan provenance¹⁴, a conclusion confirmed recently¹⁴⁻¹⁹. The importance of aeolian deposition of continental dust in islands as far afield as Barbados¹⁶ and Hawaii⁵⁰ is well documented, as is the significant aeolian contribution to deep-sea sedimentation¹⁴⁻¹⁷. Comparisons between well dated tropical cores for which ¹⁸O/¹⁶O palaeotemperature curves exist show that temperature minima coincide with high concentrations of freshwater diatoms and opal phytoliths⁵¹, as well as a large increase in the proportion of relatively unweathered clastic mineral particles^{11,52,53}. The published data show high rates of deflation and mechanical erosion during the late Pleistocene in both tropical Africa⁵¹ and Brazil¹¹.

Taken together, the evidence from pollen analysis, geochemistry, geomorphology, marine geology, and isotope chemistry is entirely consistent with low latitude aridity in Africa, India, South America and Australia during the uppermost Pleistocene. The essential problem now is to select, from among the many models^{24,29,40,41,54}, a mechanism capable of reducing effective precipitation throughout the tropics during the latter stages of an ice age.

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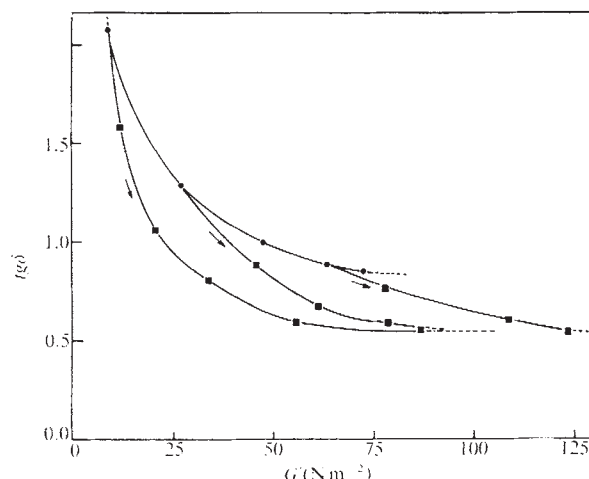
Structural hysteresis

SINCE hysteresis was first encountered in magnetism, various examples have been reported in different branches of science, as in mechanics and in surface chemistry. A unified mathematical approach to all these phenomena is possible by means of the theory of system dynamics. Such a theory shows that two time-dependent characteristics, not related by a one-to-one correspondence, will be shifted with respect to each other when they are subjected to periodic change. This phase lag causes hysteresis.

In mechanics, thixotropy is another typical example. It describes materials whose resistance to flow is lowered when subjected to mechanical agitation. In such a system the shear stress, τ , will normally not be in phase with a shear rate, $\dot{\gamma}$, that is varied periodically. Hence a τ - $\dot{\gamma}$ plot will show a hysteresis loop¹⁻³. A related, but more general, type of hysteresis in mechanics has been mentioned by Frederickson⁴.

Although thixotropy has been known⁵ for more than 50 years, no satisfactory analysis is available. Some physical measurements^{6,7} substantiate a qualitative explanation based on reversible, shear-induced changes of structure. Its application in a quantitative description has been limited to a few special cases^{8,9}. Cheng has presented a general framework for the phenomenological analysis of inelastic thixotropy in which

Fig. 1 Mechanical loss tangent as a function of shear modulus from measurements made at 0.5 Hz. ●, Equilibrium under steady state shear flow; ■, recovery under rest after shearing.



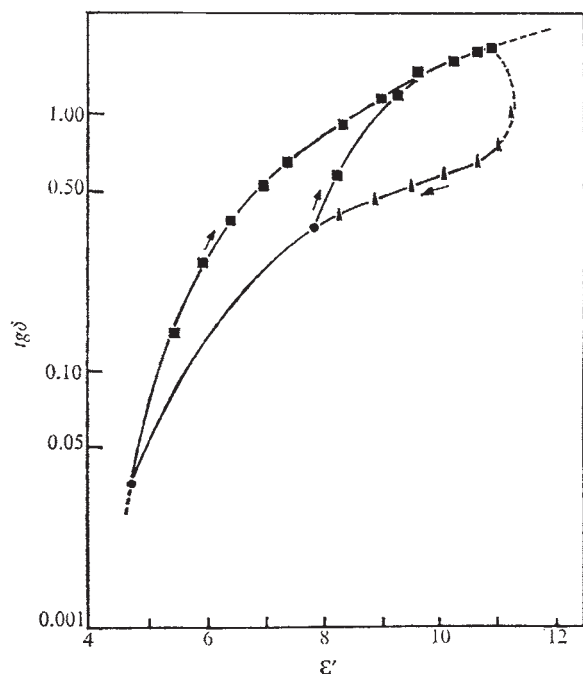


Fig. 2 Dielectric loss tangent as a function of dielectric constant from measurements made at 1.6 kHz. ●, Equilibrium under steady state shear flow; ■, recovery under rest after shearing; ▲, breakdown under shearing.

most of earlier attempts can be fitted¹⁰. Two equations are required. One is a constitutive equation relating the instantaneous value of viscosity, η , to the shear rate and to the instantaneous degree of structure through a parameter, λ ; $\eta = f(\dot{\gamma}, \lambda)$. The other is a kinetic equation relating the rate of change of the instantaneous degree of structure, $d\lambda/dt$, to the shear rate and the instantaneous degree of structure: $d\lambda/dt = g(\dot{\gamma}, \lambda)$.

An important feature of the theory is the assumption that structure can be characterised by a single parameter. Although Cheng has pointed out that this is not essential to his theory, it seriously affects the application of the theory. The general applicability of a single parameter description is of fundamental and technological importance. Yet, as far as we are aware, it has never been considered in detail. Ruckenstein and Mewis⁹ have argued against it on theoretical grounds; other investigators have done the same, using arguments based on experimental evidence^{11,12}.

Clarification is possible if several properties of the changing structure are measured simultaneously. For the one-parameter assumption to be valid, there should be a discrete one-to-one correspondence between all of them.

A mechanical investigation of thixotropic systems in the oscillatory, low frequency mode often shows a viscoelastic response^{13,14}. In that case the real and imaginary components of the modulus provide two distinct characteristics of the structure. For a chrysotile dispersion a unique relationship between both has been found in the test conditions used¹⁴. A thixotropic polyamide gel, however, revealed a deviating behaviour¹³.

We now report similar experiments using a dispersion of 3.6% carbon black in mineral oil. The dielectric properties of such a system are known to depend on structure¹⁵. Measurements in the dielectric dispersion region provide a dielectric constant with a real, ϵ' , and an imaginary, ϵ'' , part. In Figs 1 and 2 we show mechanical and dielectric data for breakdown and recovery of structure.

In both figures different paths are followed during recovery and breakdown so a single parameter theory seems inadequate for the description of thixotropy in this material. The results indicate that not only the degree of structure but also the kind of

structure depends on the shear history. We suggest that the consequent hysteresis be called 'structural hysteresis'. A quantitative discussion requires a systematic investigation of the changes in viscoelastic and dielectric spectra, which is under way and will be reported elsewhere.

But the present data indicate that not all interparticulate bonds have similar effect. As a first approximation the behaviour could be understood by assuming spherical agglomerates to be linked by particle chains in a three dimensional network. When shear is applied the interparticle bonds in the chains are more susceptible to rupture and so break down first. Subsequently, depending on the shear rate, the size of the spherical agglomerates decrease. When the shear is discontinued the more probable chain links develop first and give rise to a loose network, which slowly repairs its original structure. By changing the shear history distinct paths can be followed in plots like those in Figs 1 and 2 to go from one structure to another. We believe this is of more general kinetic and thermodynamic interest in colloid science. This picture also accounts, at least qualitatively, for some of the discrepancies in the kinetics of structural changes in thixotropic materials^{11,12}.

These results might also contribute to a better understanding of the nonlinear behaviour of other systems, such as polymers, where simplified thixotropic theories have already been applied^{16,17}.

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Dispersion of particles by shear

It is generally accepted that in the hydrodynamic dispersion of particles in liquids, shearing motions—including those produced in turbulent flows in which the viscous forces are dominant—play a central role. We draw attention here to the failure in existing theoretical treatments¹ to recognise that there are profound differences in the efficacy of different kinds of shear, and specifically between simple (or transverse) and pure (or extensional) shears.

This is illustrated by considering the two shearing flows:

$$U_1 = Gx_2 \quad U_2 = 0 \quad U_3 = 0 \quad (1)$$

$$U_1' = Gx_1'/2 \quad U_2' = -Gx_2'/2 \quad U_3' = 0 \quad (2)$$

where U_1 is particle-free liquid velocity parallel to the X_1 -axis.

The transverse shear of gradient G given by equation (1) has a rotational rate $G/2$ about the vorticity axis X_3 whereas the two dimensional flow described by equation (2), one case of extensional flow, is irrotational; the two fields, however, are identical when $X_3' (\equiv X_3)$ is rotated at $G/2$ at the instant when X_2' lags X_2 by 45° (refs 2, 3).

The simplest case of dispersion is that of a pair of rigid neutral spheres, without mutual attraction and repulsion, and with negligible Brownian motion. In field (1) they revolve in closed trajectories around one another, so that they are not mutually dispersed, as has been observed experimentally and explained theoretically⁴⁻⁷. By contrast, in field (2) (generated experimentally in a four-roller apparatus^{2,3}) we have observed with 1 mm polymethylmethacrylate (PMMA) spheres in 100 poise silicone oil that a pair of spheres always separates as predicted (P. A. Arp and S.G.M., unpublished) except when the spheres are in physical contact. This important difference results from periodic rotation of the pair in field (1) but not in field (2); for the latter flow the line joining sphere centres asymptotically approaches the X_1' direction and the separation

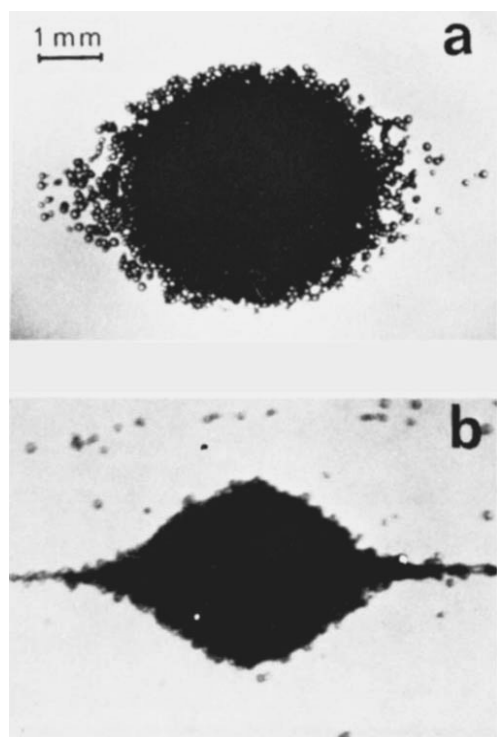


Fig. 1 Photographs showing aggregates in: *a*, simple shear flow; *b*, extensional flow at $G = 0.44 \text{ s}^{-1}$ and $t = 7 \text{ min}$. In both photographs the X_1, X_1' axes are horizontal, and the X_2, X_2' axes are vertical.

steadily increases. A similar difference in behaviour from the rotational field (1) may be expected in three dimensional (non-rotational) extension

$$U_1' = -Gx_1'/4 \quad U_2' = -Gx_2'/4 \quad U_3' = Gx_3'/2 \quad (3)$$

Another striking difference is demonstrated by a long elastomer filament. In field (1) the filament gradually coils up⁸ but the opposite happens in field (2), that is, a coil becomes extended into a straight thread aligned along the X_1' axis.

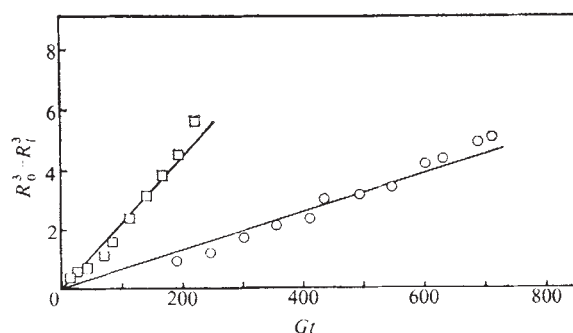


Fig. 2 Experimental test of equation (4) for aggregates like those in Fig. 1 for shear flow ($\circ$; $k = 7 \times 10^{-6} \text{ cm}^3$) and extensional flow ($\square$; $k = 22 \times 10^{-6} \text{ cm}^3$).

In simulating the dispersing process, we have carried out experiments with non-cohering aggregates consisting of concentrated suspensions, estimated at 60 volume per cent, of $100 \mu\text{m}$ PMMA spheres in silicone oil rolled into spherical balls some 3 mm in diameter. In field (1), the aggregates rotate integrally with a periodic wobble similar to viscous liquid drops of zero interfacial tension⁹, but with no sign of immediate breakup and dispersion. Instead, individual spheres from the peripheral layer gradually disengage near the regions at 45° and 225° in the direction of rotation from the X_2 axis where the extension rate is greatest. After leaving the aggregate the particles continue to circle around it and seem to be bound by the closed streamlines similar to those around a single liquid drop¹⁰. Not until this closed streamline region becomes crowded with individual particles do they start being dispersed in the surrounding fluid (Fig. 1a). At the corresponding G in the four-roller apparatus, on the other hand, the aggregates disintegrate much more rapidly with the spheres being pulled directly off the outer layers from the onset of flow and dispersed in the $\pm X_1'$ directions (Fig. 1b).

A simple analysis of the breakup, in which it is assumed that the number rate at which spheres are pulled off the periphery of the aggregate is proportional to the tensile stress generated by the sheared liquid at a given point (relative to the fixed coordinate axes) on the surface of the aggregate, leads to the following approximate relationship between the aggregate radius R_t and the shearing time t :

$$(R_0^3 - R_t^3) = kGt \quad (4)$$

Equation (2) holds reasonably well (Fig. 2) with the rate constant k , which is a measure of the dispersing efficiency, for field (1) being about 1/3 of that appropriate for field (2). This marked inferiority of field (1) is undoubtedly caused by rotation of the aggregate which causes tensile forces acting on the individual particles to reverse to compression before the particles disengage. In field (2), on the other hand, the tension (existing between $\pm 45^\circ$ of the $\pm X_1'$ axis) remains constant at a given point until disengagement occurs.

We have observed the same general behaviour with flocs of interlocked woodpulp fibres where the breakup is complicated and retarded by the periodic bending of the peripheral fibres in field (1), but which proceeds quickly, with the fibres aligning along X_1' in field (2).

Similar considerations should apply to other processes and phenomena such as: (1) fibrous crystallisation of polymers from melts and supersaturated solutions¹¹; (2) breakup of liquid drops² and the emulsification and homogenisation of liquids; (3) mixing and blending of mutually soluble polymer melts and other liquids; (4) probable breakup of erythrocyte rouleaux and other blood cell aggregates in convergent (partially extensional) flows in blood vessels. In the examples

cited, vorticity can have an inhibitory effect. This principle seems to have been realised empirically in rolling mills for rubber, inks, paints, pastry and so on, but not in any theoretical considerations.

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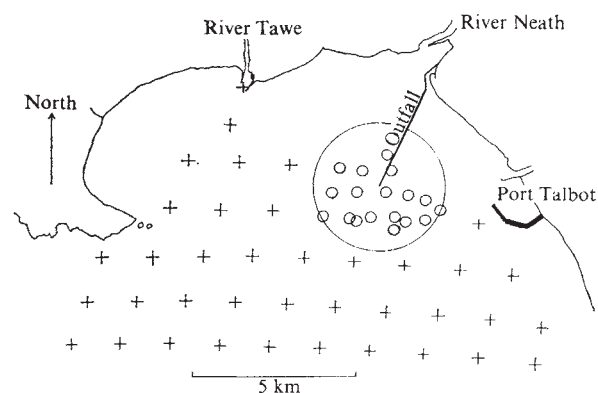


Fig. 1 Map of Swansea Bay showing sampling positions. Samples taken within a 2 km radius of the outfall are indicated as circles.

sediment type, which is determined by currents, wave action and sediment source.

Fifty-five bottom samples were taken from the bay by Shipek grab: 18 from within a 2.0-km radius of the end of the outfall, and 37 from an approximately 1.5-km grid over the rest of the bay. There was a rather higher sampling density around the outfall to enable a statistical comparison to be made between the two sets of samples. The distance of 2.0 km was chosen arbitrarily before sampling. Figure 1 shows the distribution of sampling positions. Samples were freeze dried and disaggregated before analysis, and showed no loss of mercury on freeze drying. Mercury analysis comprised digestion with aqua regia, potassium permanganate and potassium persulphate⁴, followed by flameless atomic fluorescence spectrophotometry, with a Shandon Southern mercury fluorescence unit⁵ modified to fit an Evans Electro Selenium EEL 240 atomic absorption spectrophotometer. Organic carbon was analysed by a chromic acid method, slightly modified⁶ from that of Wakley and Black⁷. Grain size analysis was done by sieving.

For the three parameters, mercury content, organic carbon, and mud fraction, the correlation coefficients were computed (Table 1). In all cases the correlations are high, and, presumably because mercury is directly associated with organic material and thus only indirectly with sediment type, the correlation between mercury and organic carbon is higher than that between mercury and mud fraction. Accordingly, the correlation between organic carbon and mercury was investigated more fully. A plot of organic carbon (percentage dry weight) against the logarithm of the mercury content (in ng per g dry weight) is reproduced in Fig. 2. The dotted line represents a fixed ratio of 250 ng mercury per g dry sediment for each percent of organic carbon. All samples from within 2.0 km of the outfall are shown as circles, and the rest as crosses. The dotted line shows that the relationship between mercury and organic carbon content for the Bay samples (crosses) approximates to simple proportionality. The mean ratio, mercury (ng per g) to carbon (%) for all samples is 283, whereas for the two populations of samples the means are 239 ($\sigma=76$) and 381

Retention of mercury from an industrial source in Swansea Bay sediments

In a study of mercury in sediments in Swansea Bay, mercury dispersed from an industrial outfall into the bay is at least partially retained in the sediments within a radius of 2 km. There is a very significant correlation between the mercury content and organic carbon content of sediments ranging from sand to mud over the whole bay, but with a higher ratio of mercury to organic carbon in the contaminated sediments.

High mercury levels in marine sediments close to sewage outfalls have been reported from California¹ and Connecticut², with the implication that these outfalls were sources of environmental mercury. There are, however, natural factors which can cause increased mercury levels in one area relative to another, and in neither study were these factors sufficiently discussed for positive conclusions to be drawn.

Thomas³ demonstrated that for freshwater sediments (Lake Ontario) there is a correlation between mercury content, organic carbon and fine grain size. We have examined the corresponding relationship for marine sediments, and deviations from the relationship which occur close to an industrial outfall.

Swansea Bay, on the South Wales coast, has very varied sediment types, ranging from clean sand with an organic carbon content less than 0.1%, to mud (90% passing through a 63 μm sieve) with 2% or more organic carbon. An outfall dispersing effluent from a chemicals complex, which includes a chlor-alkali plant, is known to release a small amount of mercury to the bay. The patchy nature of the sediment means that mercury levels vary widely from place to place, and thus isopleths of mercury contents will reflect not the influence of external sources of mercury, but

Table 1 Correlation coefficients

	Mud	All samples Organic carbon	Mercury	Mud	Bay samples only Organic carbon	Mercury	Mud	Outfall samples only Organic carbon	Mercury
Mud	—	0.91	0.69	—	0.93	0.81	—	0.81	0.50
Organic carbon		—	0.76		—	0.94		—	0.73
Mercury			—			—			—

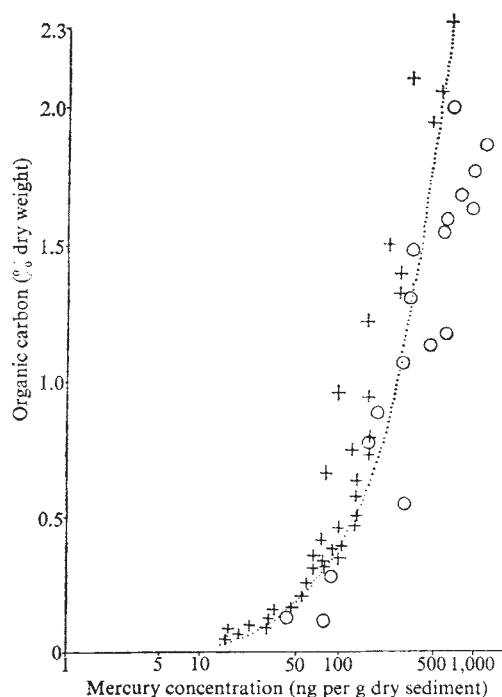


Fig. 2 The relationship between mercury and organic carbon content of the sediment samples.

($\sigma=156$) for Bay and outfall respectively. The difference is definitely significant: the difference between the sample means is more than three times the standard error of the difference⁸.

From this we conclude that some at least of the mercury from the outfall is retained in the nearby sediments. There is no other known mercury source in this area of the Bay—the River Neath, which flows in at the north-eastern corner of the Bay has very low levels of dissolved mercury (typically between 5 and 10 ng l⁻¹). Swansea Bay cannot be called heavily contaminated: the highest level found was 1,600 ng g⁻¹, which is very similar to values reported from Los Angeles¹, New Haven², Nova Scotia⁹, and Hampshire¹⁰ coastal sediments, some of which were believed to be influenced by sewage discharges. But these values are not as high as, for instance, the 9,000 ng g⁻¹ recorded in Lake St Clair¹¹, which was subjected to known mercury discharges. Thus although no serious pollution is present, the situation should be kept under observation. A detailed statistical analysis of these and other trace metal data for Swansea Bay will be published elsewhere.

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Seasonal rainfall forecasting in West Africa

To provide a basis for the seasonal prediction of rainfall in the Sahel, Winstanley¹ presented the linear regression of June on June–September rainfall for 59 stations. This regression is statistically inappropriate. June rainfall should be taken as the independent variable and not included in both the dependent and independent variables. Though June rainfall accounts for less than 15% of the seasonal rainfall on average, it accounts for a much larger proportion of the variance. The coefficient of

Table 1 Regressions of seasonal rainfall (Y) on monthly rainfall (X), both expressed as % departures from the mean (based on 1931–60 wherever possible)

	Correlation coefficient	Significance level
(a) $Y = -6.0 + 0.129X$	0.22	$P < 0.40$
(b) $Y = -5.2 + 0.251X$	0.44	$P < 0.06$
(c) $Y = -7.2 + 0.291X$	0.64	$P < 0.01$
(d) $Y = -5.8 + 0.327X$	0.41	$P < 0.06$
(e) $Y = -3.3 + 0.239X$	0.26	$P < 0.05$

- (a) 50 stations between 10° and 20° N, 1953–1972, using July to September on June.
- (b) 50 stations between 10° and 20° N, 1953–1972, using June to September on June.
- (c) 59 stations between 10° and 20° N, 1953–1972, using June to September on June (from Winstanley¹).
- (d) 50 stations between 10° and 20° N, 1953–1972, using August to September on July.
- (e) 5 stations between 11° and 14° N, 1905–1973, using August to September on July.

variation for June rainfall on the southern edge of the Sahel is about 50% but for the total rainfall it is only about 20%.

The simplest predictor would be the regression of July–

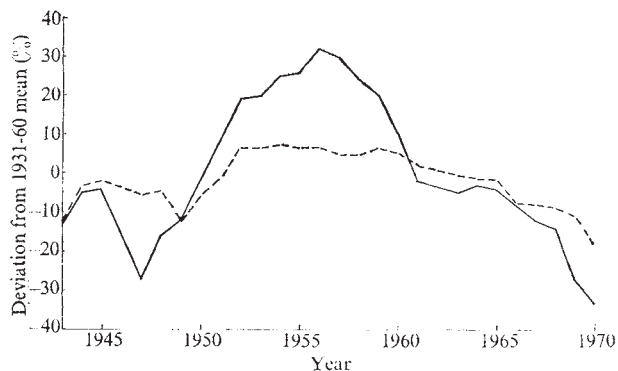


Fig. 1 5-yr running means of annual rainfall as percentage of 1931–60 mean for five stations with mean latitude 18° 28' N (ref. 2) (—), and for the five stations used here with mean latitude 12° 48' N (---).

Table 2 Contingency table for July and August–September rainfall based on 5 stations between 11° N and 14° N for the period 1905–73

		August–September		
		Wet	Normal	Dry
July	Wet	8	11	4
	Normal	11	4	8
	Dry	4	8	11

$$\chi^2 = 9.65 \quad P < 0.05.$$

Table 3 Correlation coefficients between Niamey 700 mbar mean monthly geopotentials and mean rainfall for five stations between 11° N and 14° N, 1953–72 (probability in brackets)

	June	July	Monthly and seasonal rainfall		July and August	July to September	August and September
			August	September			
Niamey 700 mbar geopotential							
June	-0.353 (20%)	-0.316 (20%)	-0.445 (6%)	-0.305	0.475 (5%)	-0.317 (20%)	-0.200
July		-0.464 (5%)	0.637 (1%)	-0.183			0.443 (6%)
August			-0.781 (0.1%)	0.008			
September				-0.053			

September rainfall on June rainfall. This regression cannot be reconstructed from Winstanley's graph. But an examination of 50 stations in the same area and for the same period, 1953–72, showed no statistically significant correlation (Table 1). If June rainfall is included in the dependent variable there is a linear correlation, similar, to, but statistically less significant than, that calculated by Winstanley¹. Individual monthly rainfalls were not significantly correlated except for July and August ($r = 0.44$, $P < 0.06$). The regression of August and September rainfall on July rainfall was also statistically significant (Table 1).

A similar analysis for a longer period can be made by using fewer stations. For five stations with relatively long records (Kano, Maiduguri, Sokoto, Niamey and Zinder) departures of rainfall from the 1931–60 normals were calculated and averaged for each year for which records were available in the period 1905–73. The departures at these stations, which are on the southern edge of the Sahel, are similar in pattern to those calculated for five more northerly stations² (Fig. 1). For these southern stations, July and August rainfall ($r = 0.23$, $P < 0.05$) and August and September rainfall ($r = 0.29$, $P < 0.02$) are the only pairs of months to show significant correlation.

The August and September rainfall total is significantly correlated with July rainfall (Table 1). The confidence limits for this regression are large and a useful summary of the observations is obtained by dividing them into terciles and constructing a 3 × 3 contingency table (Table 2). The use of a contingency table emphasises that only a general indication of the amount of rainfall to come can be given. A precise forecast cannot be made.

These results show that West African rainfall has some seasonal persistence, at least from July onwards. Another effect which is probably related was shown by Delsi³ who examined mean monthly geopotentials over the Sudan and West Africa. He found that the mean monthly 700 mbar geopotential anomaly in both areas tended to persist from June until August. Persistence was less evident for earlier months. He also investigated the relationship between the June 700 mbar geopotential anomaly at Khartoum and seasonal (July and August) rainfall for the Sudan for the years 1953 to 1968. The correlation coefficient with rainfall amount was -0.49 ($P < 0.06$) and with number of days with greater than 10 mm of rain was -0.65 ($P < 0.01$).

We have examined the relationships between the Niamey 700 mbar mean monthly geopotential and the average rainfall for the five stations over the period 1955–73. The geopotentials in successive months, between June and September are significantly correlated, with correlation coefficients greater than 0.8 ($P < 0.001$). Correlation coefficients between the geopotentials and monthly and seasonal rainfalls are given (Table 3).

For West Africa, seasonal persistence is apparent in the 700 mbar geopotential anomaly from June to September and in rainfall from July to September. As a possible predictor the variations of geopotential in the region itself are likely to be more appropriate than those at 40° N quoted in a general way by Winstanley² or Bryson⁴. Useful seasonal forecasts seem possible. We plan to test such forecasts further in cooperation with the Meteorological Office.

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Possible mechanism for discrimination between nitrate and nitrite by nitrate reductases

RECENT studies¹ have been concerned with the mechanism of redox reactions between nitrate ions and simple mononuclear oxomolybdenum(V) (Mo^{VO}) complexes such as $[\text{MoOCl}_3(\text{OPPh}_3)_2]$ to determine the function of the molybdenum centre in the nitrate reductase enzymes. In the presence of excess nitrate, the following reaction stoichiometry is obtained:



Kinetic studies using stopped-flow techniques identified three stages in these reactions: (1) the loss of the ligand *trans* to the oxo-group of the Mo^{VO} complex followed by rapid, non-rate-determining coordination of nitrate at the vacated site; (2) a rearrangement of the Mo^{VO} -nitrate-complex to produce a geometry suitable for rapid electron transfer resulting in the formation of NO_2 and a *cis*-dioxomolybdenum^{VI} (Mo^{VIO_2}) complex; (3) ligand substitution(s) at the *cis*- Mo^{VIO_2} centre.

Nitrite is considered to be a better nucleophile than nitrate² and redox potential data³ suggest that nitrite should be an oxidant comparable with nitrate. These facts contrast markedly with the exclusive reduction of nitrate by the nitrate reductase enzymes, even though nitrite seems to be able to coordinate to a $\text{Mo}(\text{V})$ centre in these enzymes⁴. We have therefore examined the mechanism of the reaction between $[\text{MoOCl}_3(\text{OPPh}_3)_2]$ and Et_4NNO_2 in CH_2Cl_2 at temperatures from 0 to 25° C for direct comparison with the nitrate oxidation described above. Frank and Spence⁵ have shown that in aqueous solution, $\text{Mo}(\text{V})$ complexes will quantitatively reduce nitrite to NO and we also found this in our system. The non-aqueous system we used provided new information concerning the mechanism of nitrite reduction by a $\text{Mo}(\text{V})$ centre which, when compared to the corresponding reduction of nitrate, suggests possible

ways in which nitrate reductases discriminate between these oxidants.

In the presence of excess nitrite, the reaction between $[\text{MoOCl}_3(\text{OPPh}_3)_2]$ and Et_4NNO_2 proceeds according to equation (2) in three stages,



the mechanism of which seems to resemble closely those described above for the corresponding reaction with nitrate. Consistent with the S_N1 (limiting) mechanism proposed for the first stage of this reaction, the rate-determining step involving the loss of the Ph_3PO molecule *trans* to the oxo-group, has a rate constant for nitrite substitution at 25°C of $52 \pm 2 \text{ s}^{-1}$ which is very similar to that obtained ($40 \pm 1 \text{ s}^{-1}$) for the corresponding nitrate substitution. Kinetic data obtained for these substitutions, carried out in the presence of an excess of Ph_3PO , show that the nucleophilicity of these ligands towards the five coordinate intermediate $[\text{MoOCl}_3(\text{OPPh}_3)_2]$ varies in the order $\text{NO}_2^- > \text{NO}_3^- \gg \text{Ph}_3\text{PO}$ as 47 : 31 : 1. This difference between the affinity of the two anions for the Mo(V) centre, together with the general preference for nitrite to function as an N-donor ligand⁶ support a tentative assignment of $[\text{MoOCl}_3(\text{OPPh}_3)(\text{NO}_2)]^-$ as the nitro-complex *a* (Fig. 1) rather than the corresponding nitrito-complex. The kinetic data obtained for the second stage of the reaction between $[\text{MoOCl}_3(\text{OPPh}_3)_2]$ and Et_4NNO_2 indicate that the rate of this process (k_2) is independent of the concentrations of Mo^{V} , Et_4NNO_2 and Ph_3PO . At 25°C $k_2 = 2.6 \pm 0.5 \times 10^{-2} \text{ s}^{-1}$ and the process is considered to involve the intramolecular rearrangement of *a* to *b* (Fig. 1), a process similar to that suggested¹ for the corresponding stage of the nitrate reaction although this proceeds at a much faster rate, k_2 (25°C) = $1.0 \pm 0.2 \text{ s}^{-1}$. A comparison of the ultraviolet spectra obtained (C.D.G., P. M. Boorman, P. Lambert, F.E.M., and V. I. Routledge, unpublished) for Mo(V) and Mo(VI) complexes suggests that the final product of the second stage of the nitrite reaction is a Mo(VI) species. Therefore, after the rearrangement k_2 , a fast electron transfer process, $k_{\text{et}} \geq 5 \times 10^{-2} \text{ s}^{-1}$ at 25°C , is considered to occur, producing *c* (Fig. 1) plus NO . The latter was collected as a gas, identified by infrared spectroscopy⁷ and quantitatively estimated by condensation in to acidified KMnO_4 solutions, followed by back titration with Fe(II) (ref. 8). The yields of NO were 77–105% of those predicted from equation (2) based on Mo(V) . The changes in the absorbance spectrum

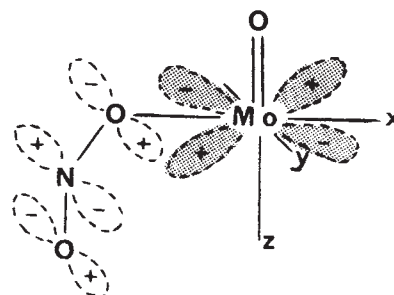


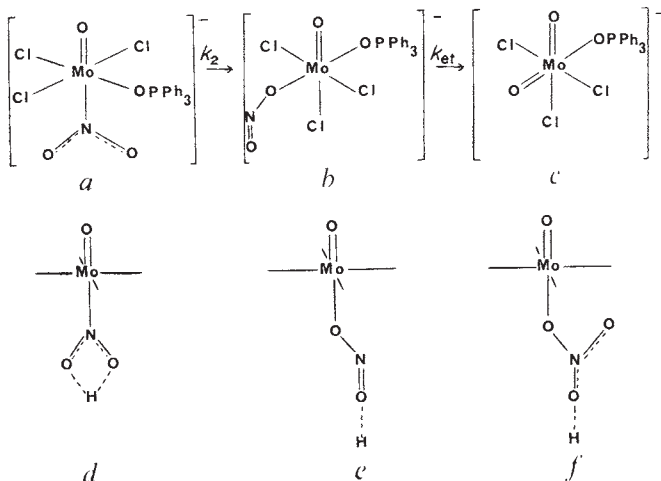
Fig. 2 Desired orbital overlap for reduction of nitrite to NO by the $\text{Mo}^{\text{V}}\text{O}$ centre.

observed during the third stage of the reaction between $[\text{MoOCl}_3(\text{OPPh}_3)_2]$ and Et_4NNO_2 were consistent with ligand substitution processes of Mo(VI) complexes.

The results of this and the previous study¹ suggest that electron- and oxygen-transfer processes between Mo(V) and nitrogen oxanions are interrelated and require a specific atomic arrangement of the reactants. The criteria developed¹ for nitrate oxidation of a Mo(V) centre seem to apply equally well to nitrite oxidation of this entity. Molecular orbital calculations⁸ for the nitrite ion indicate that the π^* orbital is the lowest energy virtual orbital. Therefore, the most favourable route for electron transfer would be expected to occur when this orbital overlaps with the highest energy (partially) filled orbital on the metal, $4d_{xy}$ (ref. 10 and C.D.G., I. H. Hillier, and F.E.M., unpublished), providing that electron transfer can lead to the ready formation¹¹ of stable Mo(VI) and N(II) species. Such criteria seem to be exclusive to the arrangement where a nitrito-group is bound to Mo(V) by way of one oxygen atom coordinated *cis* to the oxo-group (Fig. 2). This atomic arrangement affords both the desired orbital overlap together with a route for the ready formation of a *cis*- $\text{Mo}^{\text{VI}}\text{O}_2$ moiety and NO .

If the reactive site for nitrate reduction in the nitrate reductases is a mononuclear $\text{Mo}^{\text{V}}\text{O}$ (or $\text{Mo}^{\text{IV}}\text{O}$) centre the site for the easiest initial binding of the substrate would be expected to be *trans* to the oxo-group. The substrate is probably introduced at this position by favourable hydrogen-bonding to a suitably placed X-H group of the protein; differences in hydrogen bonding perhaps accounting for the different affinities for nitrate exhibited by the various nitrate reductases (E. J. Hewitt, unpublished). It is unlikely, however, that such a site *trans* to the oxo-group could exercise much selectivity for nitrate over other small anionic ligands in general, and nitrite in particular. The coordination of nitrate, nitrite, and azide to the Mo(V) centre suggested by electron spin resonance⁴ studies is consistent with this view. A clear possibility for selective reduction of nitrate over nitrite, however, does exist if, before electron- and oxygen-transfer, the substrate has to be coordinated to the molybdenum by way of one oxygen atom *cis* to the oxo-group. A comparison of the $[\text{MoOCl}_3(\text{OPPh}_3)_2]$ reductions of nitrate and nitrite shows that such reorganisation proceeds some 38 times faster for the former. We suggest that the rearrangement is relatively difficult for nitrite in this model system because it prefers to coordinate as an N-donor ligand⁶. The intramolecular reorganisation (which presumably involves a nitro- to nitrite-isomerisation, the reverse of that reported¹², followed by (O,O) chelation) required to proceed from *a* to *b* (Fig. 1) will be considerably greater than that for the corresponding nitrate-complex (compare fig 1f) which has an oxygen atom more suitably situated for the required attack. In the nitrate reductase enzymes, it is possible that hydro-

Fig. 1 Simple mononuclear $\text{Mo}^{\text{V}}\text{O}$ complexes. Intramolecular reorganisation during nitrate and nitrite reductions. For explanation, see text.



gen bonding of the substrate could enhance this discrimination. The process could be made less favourable for nitrite, either by making the *cis* coordination of an oxygen atom of the nitro-ligand more difficult (Fig. 1d), or by stabilising nitrito-coordination (such coordination could also be favoured by some steric restrictions at the metal site⁶), in which the uncoordinated oxygen atom is held back from the *cis* sites (Fig. 1e). This latter model is favoured over the former since a nitrate-ligand could fit directly into the site of *e* and yet have an oxygen atom poised for attack at a site *cis* to the oxo-group (Fig. 1f). In this manner the reduction of nitrate could readily proceed, whereas that of nitrite could not.

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Ubiquinone-O in defensive spray of African millipede

WHEN strongly disturbed, the red-legged millipede *Metiche tanganyicense* Kraus (Spirobolida: Trigonulidae) sprays attackers with a mixture of four quinones (Fig. 1). Ubiquinone-O, a major component of this defensive spray has not been isolated previously from a natural source.

These millipedes were easily collected as they sought refuge in human habitation during the rainy season at Mombasa, Kenya. When roughly handled, the millipede would propel its orange-red defensive secretion about 40 cm. The secretion tans skin and is quite irritating to a mucous membrane; it gives a positive response for *p*-quinones with rhodanine and aqueous ammonia¹.

To collect the defensive spray each millipede was placed in a glass tube and stimulated electrically until it discharged its secretion. Thereafter the millipede was removed from the tube and any secretion adhering to its body was collected on glass wool. Both the glass tube and the glass wool were washed with ether. Gas chromatographic analysis of this solution indicated that four volatile components were present.

Two of these components were readily identified as the common millipede quinones; toluquinone (Fig. 1, I) and 2-methoxy-3-methylbenzoquinone (Fig. 1, II)². The infrared spectra, mass spectra and gas chromatographic retention time of these components were identical to those of authentic samples.

The third component gave a positive test for a *p*-quinone with rhodanine and aqueous ammonia¹. Its infrared spectrum in CHCl₃ further substantiates a quinoidal structure, showing a strong carbonyl absorption at 1,654 cm⁻¹ and an olefinic absorption at 1,584 cm⁻¹. A dimethoxybenzoquinone

structure (C₈H₈O₄) is suggested by a mass spectral molecular ion at *m/e* 168. The nuclear magnetic resonance (NMR) spectrum in CDCl₃ (values, δ , for chemical shifts in p.p.m.) confirm this, with two olefinic protons (6.68, singlet) and six methoxy protons (4.03, singlet). As this compound has a melting point of 66–67° C it must be 2,3-dimethoxybenzoquinone (Fig. 1, III) (melting point, 66–67° C (ref. 3)) the melting points of the 2,5-isomer and the 2,6-isomer are 250° C and 254–255° C respectively⁴. This quinone has been previously characterised in the defensive secretion of the millipede *Uroblaniulus canadensis*⁵.

Finally, the component of longest gas chromatographic retention time which also gave a positive colour test for a *p*-quinone¹, showed infrared absorptions typical of a and an olefinic absorption at 1,601 cm⁻¹ (CHCl₃). A dimethoxymethylbenzoquinone structure (C₉H₁₀O₄) is indicated by xymethylbenzoquinone structure (C₉H₁₀O₄) is indicated by the observation of a molecular ion at *m/e* 182. The NMR spectrum (CDCl₃) of this compound confirms this, showing an olefinic proton (6.45, quartet: *J*=1.5 Hz), three methoxy protons (4.05, singlet), three methoxy protons (4.02, singlet)

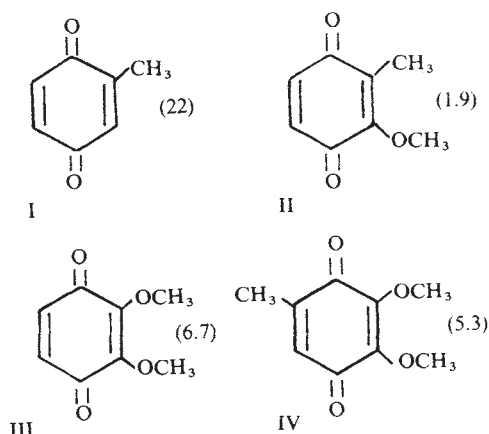


Fig. 1 The quinones from *M. tanganyicense* with the amount of each (mg) from a millipede (8 g).

and three methyl protons (2.04, doublet: *J*=1.5 Hz). As the olefinic proton and the methyl protons are coupled the compound must be 2,3-dimethoxy-5-methylbenzoquinone (Fig. 1, IV) (ubiquinone-O). Published NMR⁶ and mass spectra⁷ of IV confirm this assignment.

While spraying of defensive secretions by millipedes is not unknown, discharge from oozing glands is more usual⁸. We have examined anatomically and histologically the glands of *M. tanganyicense* and find them to be very similar to those described by Woodring and Blum for the spraying millipede *Orthocricus aboreus*⁹.

Undoubtedly, the red legs of *M. tanganyicense* warn predators that it is different from the other millipede common in the area, *Archispirostreptus gigas*, which is entirely dark brown and has oozing glands that secrete a mixture of toluquinone and 2-methoxy-3-methylbenzoquinone¹⁰. The difference between these two millipedes was learned by the dwarf mongoose *Helogale parvula* on its initial encounter with *M. tanganyicense*: one spraying was sufficient to discourage further attacks on this millipede (P. Robins, unpublished). *A. gigas*, which does not spray its secretion, was readily eaten by the same mongoose. On the other hand, the elephant shrew *Rhynchocyon chrysopygus*, which inhabits the coastal forests of Kenya, eats *M. tanganyicense* and does not seem bothered by its defensive secretion (G. Rathbun, unpublished).

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Piezoelectric properties of bone as functions of moisture content

FUKADA and Yasuda¹ showed that dry bone is piezoelectric in the classic sense, that is, mechanical stress produces polarisation (direct effect) and application of an electric field produces strain (converse effect)². The possibility that the mechanism for osteogenesis is electrical³ has aroused interest in an investigation of such effects in the more nearly physiological condition represented by wet bone. Recent developments in electrical stimulation of fracture healing^{4,5} have further emphasised the importance of characterising the piezoelectric properties of wet bone. As the piezoelectricity of wet collagen⁶ decreases to zero at a moisture content equal to 45% of the dry weight (which corresponds to almost 100% humidity)⁷, there is some doubt as to whether wet bone could be piezoelectric. Observations of voltages in wet bone under stress, have been made under conditions giving such ambiguous results that they suggested alternative concepts to that of classic piezoelectricity⁸⁻¹¹. In view of the confusion about the nature of the voltage developed when wet bone is stressed, we have used a simple longitudinal stress system with sinusoidally varying drive, to measure both converse and direct piezoelectric coefficients of bone as functions of humidity (and ultimately of moisture content). The results show unambiguously that wet bone behaves as a piezoelectric material.

Bone was cut from adult human femur, and stored in Ringer solution at 3°C for several weeks. Samples (4.2 mm³) were cut in the two orientations shown in Fig. 1, that is parallel to the three principal axes, and rotated 45° about the *x* axis. Silver epoxy electrodes were attached to the endosteal and periosteal surfaces (perpendicular to the *x* axis) for the 45° specimens and perpendicular to the bone axis for the unrotated specimens.

A test apparatus similar to Fukada's¹² was used with some modifications so that both converse and direct effect could be measured. These included additional shielding and loading the bone and quartz outputs with a large capacitor, so that a charge amplifier was used to measure direct effect. A bone specimen was clamped together with a quartz crystal cube and a ceramic disk of high piezoelectric coefficient (PZT5). The PZT5 ceramic served to drive the system for the direct effect, and acted as the strain detector for the converse effect. The quartz crystal was used as a standard.

The frequency (in the range 3,200 to 5,600 Hz) was chosen so that the system was at resonance, to increase the sensitivity and to reduce the effect of pickup. By keeping the test apparatus in a sealed chamber containing an appropriate saturated salt solution, and immersing the chamber in a constant temperature water bath, both temperature and humidity were controlled. One week was allowed for equilibration at each humidity.

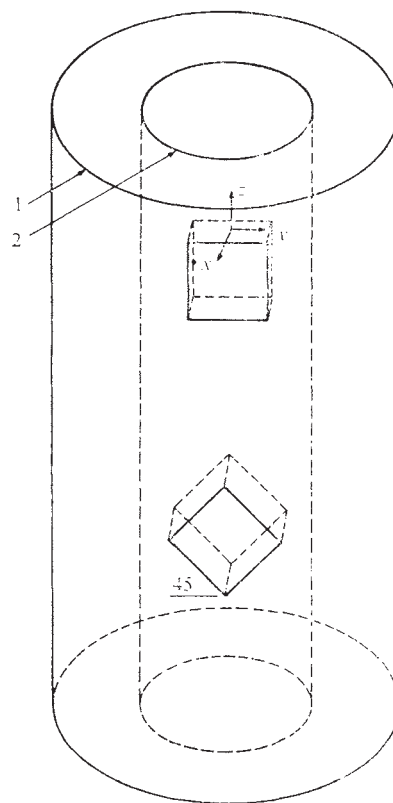


Fig. 1 Location of bone specimens with respect to femur. 1, Periosteal surface; 2, endosteal surface.

From work on dry bone¹, we know that the principal piezoelectric coefficient is the quantity d_{14} , which involves coupling between an electric field in the *x* direction and a shear strain in the *yz* plane. The coefficient d_{14} is most easily obtained from the specimen rotated 45° about the *x* axis of the bone (Fig. 1), using longitudinal strain. Experiments carried out with the sample cut parallel to the *x* axis yielded the coefficient d_{31} (that is, electric field or polarisation in the *z* direction).

The variation of d_{14} with humidity (obtained from both converse and direct effects) is shown in Fig. 2. The agreement obtained between the measurements of converse effect and direct effect is quite good; we believe the small systematic disagreement is experimental in origin. Comparison of data taken in the direction of increasing humidity with those taken with decreasing humidity shows, however, that the measurements display a hysteresis effect, that is, the piezoelectric coefficient is a function of previous hydration history as well as of relative humidity. Recognis-

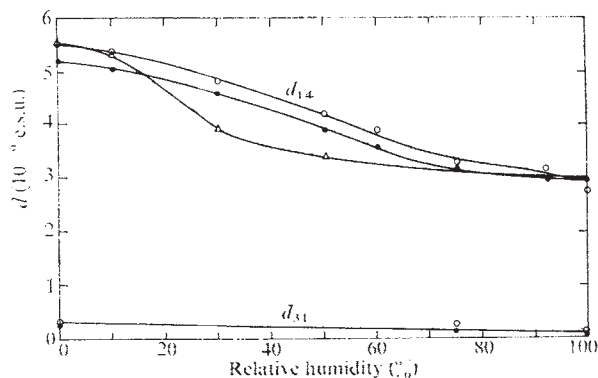


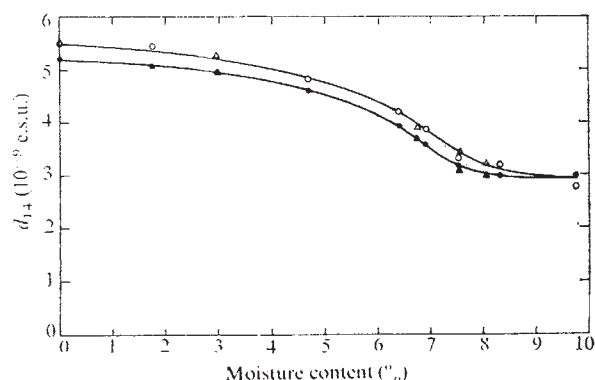
Fig. 2 Piezoelectric coefficients of bone as functions of relative humidity measured at 5,600 Hz. d_{14} , Converse effect, dry to wet ($\circ$); direct effect, dry to wet ($\bullet$); converse effect, wet to dry ($\triangle$). d_{31} , Converse effect, dry to wet ($\circ$), direct effect, dry to wet ($\bullet$).

ing that moisture content (that is, weight of water per unit weight of dry bone) represents a more fundamental variable than humidity, we determined the moisture content of similar samples of bone as a function of relative humidity by direct weight-change measurements over several wetting and drying cycles¹³. These measurements were used to convert the piezoelectric data from functions of humidity to functions of moisture content. The piezoelectric coefficient d_{14} is shown plotted in this way in Fig. 3, which shows that as a function of moisture content the data give single-valued behaviour, with no hysteresis, that is, the results are independent of past history. (Figure 3 also includes data for the direct effect in the direction of decreasing humidity, which have been omitted from Fig. 2 for the sake of clarity.)

From a comparison of the present results with the variation of d_{14} of collagen with moisture content, it follows that at 100% humidity the collagen in bone must have a moisture content of 12%, only slightly higher than the average moisture content of bone itself. The moisture uptake of free collagen⁶ and of collagen in bone differs probably because the latter is intimately bonded to the mineral phase and is not free to swell.

Using a specimen cut parallel to the bone axes, we measured the piezoelectric coefficient d_{31} . As Fig. 2 shows, the values of d_{31} are an order of magnitude lower than d_{14} , but decrease with increasing moisture content in the same way. This result is in direct contradiction to the work of Anderson and Eriksson⁹ who observed increasing values of d_{31} for wet bone, and attributed these results to streaming potentials. Our results show no evidence to support the streaming-potential concept. Rather, it can be stated that for sinusoidal drive at audio frequencies, bone behaves in a classic piezoelectric manner, with good agreement between converse and direct effect, at all humidities.

Fig. 3 Coefficient d_{14} of bone as function of moisture content: converse effect, dry to wet ($\circ$); direct effect, dry to wet ($\bullet$); converse effect, wet to dry ($\triangle$); direct effect, wet to dry ($\blacktriangle$).



The following paper¹⁴, demonstrating that both bone and tendon are piezoelectric in the hydrated, though frozen, state, is consistent with our findings.

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Piezoelectricity in hydrated frozen bone and tendon

THE piezoelectric property of connective tissue may play a role in regulating the patterns of tissue growth¹. Most piezoelectric measurements, however, deal with dried tissue²⁻⁴. Observations of stress-generated voltages from hydrated connective tissue are generally insufficient to establish that the voltages are of piezoelectric origin, because of complications resulting from streaming potentials and electrode effects⁵⁻⁷. A report of the non-existence of piezoelectricity in hydrated collagen at room temperature⁸ has been criticised⁹. Use of the converse effect is most desirable in studying biological piezoelectricity⁹, but for hydrated tissue the high electrical conductivity of water interferes with the establishment of an electric field inside the sample^{2,4}. We therefore hydrated and then froze our connective tissue samples taking advantage of the reduced conductivity of ice compared with that of water. Our results establish the existence of the piezoelectric effect in bone and tendon under physiological conditions of moisture, but at a non-physiological temperature (-25°C).

We used femurs and Achilles tendons of cows, aged 3 to 4 yr when slaughtered. Samples approximately $10 \times 5 \times 3$ mm, orientated to display the piezoelectric coefficient d_{14} , were prepared and measured by the converse effect.

Measurements on dry bone and tendon were made at 24°C after drying the samples at 100°C for 24 h. Samples were fully hydrated by immersing in saline at 24°C for 24 h then after hydration, they were frozen at -25°C for 24 h and then measured at -25°C . The small dimensional changes in bone following hydration were ignored, whereas the corresponding changes for tendon were not negligible and therefore the dimensions of the frozen tendon samples were used to compute their hydrated piezoelectric coefficients.

The results (Table 1) show that hydrated frozen bone and tendon are piezoelectric. The effect of both freezing and hydrating is to reduce the magnitude of d_{14} by about a factor of two for bone and a factor of eight for tendon. About half of the decrease for tendon can be accounted for on the basis of the volume increase which accompanies hydration.

Whatever structural changes result from the incorporation

Table 1 Piezoelectric coefficients (d_{14}^*) and standard deviations of bovine bone and tendon.

	Dry (at 24° C)	Hydrated (at -25° C)
Bone	5.45 ± 0.82 (22)	2.9 ± 0.6 (7)
Tendon	86.6 ± 18.4 (4)	10.2 ± 2.8 (7)

Numbers in parentheses indicate number of samples measured.
 $^* \times 10^{-9}$ c.g.s. e.s.u.

of water by collagen, the piezoelectric property is relatively unaffected at -25° C. In view of the marked stability of collagen as determined by piezoelectric measurements^{4,10}, it seems unlikely that an increase to physiological temperatures would produce structural changes so drastic as to destroy the piezoelectric property. The preceding paper¹¹ presents evidence which supports this contention.

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Ultraviolet light activation of insect nuclear polyhedrosis virus

IRRADIATION within the ultraviolet (190.0-380.0 nm) range is an effective means of virus inactivation. We report the effects of short exposures of ultraviolet light at 320.0 nm on *Autographa californica* (Lepidoptera) nuclear polyhedrosis virus. In this case, ultraviolet light has an activating effect on the virus produced *in vitro*.

An aqueous suspension of *Trichoplusia ni* nuclear polyhedrosis virus inclusion bodies seems to be completely inactivated when exposed to 253.7 nm ultraviolet light at an incident distance of 15 cm for 1 min¹. Gudauskas and Canerday² exposed partially purified suspensions of *T. ni* nuclear polyhedrosis virus and *Heliothis* nuclear polyhedrosis virus to 253.7 nm ultraviolet light at a distance of 2-5 cm, and reported apparent total inactivation of the *Heliothis* virus after 5 min at 2 cm and almost total inactivation of the *Trichoplusia* virus by 10 min at 2 cm. They noted a decrease in ultraviolet inactivation as the incident distance increased. Bullock *et al.*³ exposed purified suspensions of *Heliothis* polyhedra to wavelengths of 253.7, 307.5 and 364 nm at a distance of 10.2 cm, and found significant inactivation of virus in the 253.7 and 307.5 nm range. They suggested that at 364 nm some virus reactivation may have occurred. Witt⁴ studied the photochemistry of *Galleria mellonella* nuclear polyhedrosis virus and found that non-occluded nucleocapsid suspensions were inactivated when exposed to ultraviolet light of 253.7 nm at 50 cm and 320.0 nm at 25 cm. The near ultraviolet (320.0 nm) did not increase virus activity.

Immediately after exposure of the virus samples (prepared as in Fig. 1) to ultraviolet light, larval inoculation was performed. For each sample, twenty larvae were intracoelomically inoculated with 0.5 μ l per larva. Twenty larvae were injected with a blank sample containing only distilled water. The *in vitro* plaque assay of the irradiated suspensions showed that,

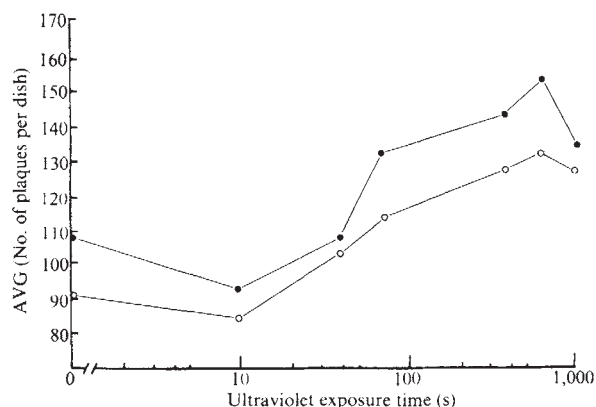
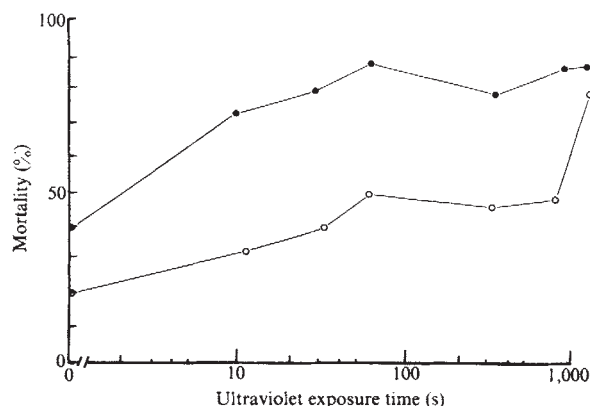


Fig. 1 Plaque assay of *A. californica* nuclear polyhedrosis virus, irradiated with ultraviolet (UV) light on TN-368 cells. Each line represents a replication. Each dot is the average of two plaque counts. Polyhedra-free nucleocapsid suspensions were assayed *in vitro* using a plaque assay technique⁶ on the *Trichoplusia ni* (TN-368) cell line. The suspensions were also bioassayed on 7-d-old *T. ni* larvae. The viral inoculum was obtained by infecting the TN-368 cell monolayers with *A. californica* nuclear polyhedrosis. Cells from these infected monolayers were centrifuged at 164g, resuspended and washed twice in Hanks' phosphate buffered saline⁷. The saline was drawn off and the cells resuspended in distilled water. This procedure lysed the cells and the suspension was spun at 1500g for 20 min. The supernatant was filtered through a 0.45 μ m Millipore filter. This was the stock preparation. Plaque assays were performed on serial tenfold dilutions of this preparation for quantitating plaque forming units (PFU). Similarly, the LD₅₀ of the virus preparation was determined by bioassaying serial tenfold dilutions by injection into 7-d-old *T. ni* larvae. The virus preparation was diluted to approximately 10⁴ PFU ml⁻¹ in sterile distilled water. Aliquots (1 ml) were pipetted into depressions (2 cm diameter) of plastic depression trays (Limbro Chemical Co., Inc.) and exposed to two General Electric BLB fluorescent black lights (output 15 W at 320.0 nm) at 22 cm. The samples were exposed for time periods of 0 to 17 min and were gently agitated during exposure. After exposure, the suspensions were diluted to the titre which would allow the assays to be read.

rather than inactivation, virus activation seems to have occurred (Fig. 1). A comparison of plaque numbers of the three shortest irradiation periods (0, 10 and 30 s) with the three longest irradiation periods (300, 600 and 1,020 s) using an unpaired *t* test⁸ revealed a difference between means that was significant at the 98% level (*t* = 2.89). An activation mechanism would seem to be responsible for this significant increase in plaque forming units. The results of the larval bioassay showed a marked increase in virus activity as ultraviolet exposure time increased (Fig. 2). This represents approximately a 200-fold increase in virus activity when the LD₅₀ values are calculated.

Fig. 2 Larval (8-d-old *Trichoplusia ni*) bioassay of *A. californica* nuclear polyhedrosis virus irradiated with ultraviolet light. Each line represents a replication.



These data show that near ultraviolet light has an activating effect on *A. californica* nuclear polyhedrosis virus produced *in vitro*. The two assays demonstrate that the effect is related to radiation dosage. The larval bioassay suggested that most activation occurred during the first 60 s and the plaque assay that the effect began at 60 s and lasted for 420 s. This may result from differences in the sensitivities of the two assay systems.

Ultraviolet light enhanced by reactivation and photo-reactivation occurs in mammalian viruses⁹, avian viruses¹⁰ as well as plant viruses¹¹. We present the first account of near-ultraviolet light induced activation of an insect nuclear polyhedrosis virus. Further investigation will be needed before the mechanism involved in this activation can be determined.

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Olfactory response in excitable protoplasmic droplet and internodal cell of *Nitella*

In higher vertebrates, olfactory reception takes place at the receptor cell in the olfactory epithelium. The elicitation of neural responses caused by odorants is not restricted to the olfactory organs; other excitable systems (for example, the trigeminal nerve and vomeronasal organ of the tortoise^{1,2}, ganglion cell³ of *Helix* and contact chemoreceptor of the fly⁴) are also to respond to odorants. Excitable membranes in general probably respond to odorants, and the processes of olfactory reception and of excitation of a membrane must be closely related. The interaction between odorants and an excitable membrane which is basic to the molecular process of olfactory stimulation, has not been investigated with this relationship in mind, because of the small size of the olfactory cells and of the excitable systems mentioned. This factor has prevented the recording of the intracellular receptor potential in response to odorants. To clarify the physicochemical mechanism underlying the olfactory reception, we have therefore used the protoplasmic droplet and internodal cell of *Nitella* to investigate the effect of odorants on the electrical properties of an excitable membrane. Apart from its size, the protoplasmic droplet has the advantage of having a functional membrane contiguous with the external solution⁵⁻⁷.

An internodal cell of *Nitella flexilis*, immersed in a solution containing 0.5 mM CaCl₂ and 300 mM mannitol with no univalent cation species, has a resting potential of about -100 mV and an action potential of 50-60 mV (about 30 s long) after electrical stimulation. The excitable protoplasmic droplet was prepared as previously described^{8,9}. The internodal cell was amputated and the effused protoplasmic droplet allowed to stand in a salt solution (0.5 mM NaNO₃, 0.5 mM KNO₃, 1 mM CaCl₂, and 2 mM MgCl₂).

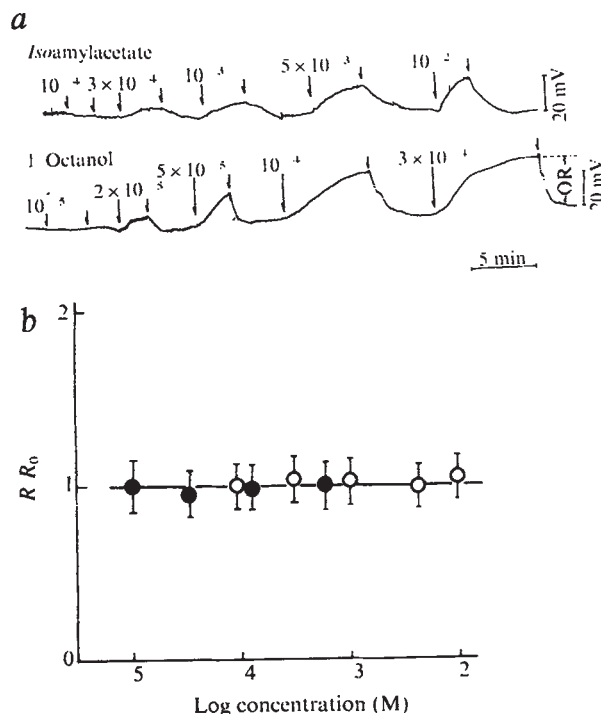


Fig. 1 Variations in the membrane potential and the electrical resistance observed for a protoplasmic droplet in response to various concentrations of isoamylacetate (○) and 1-octanol (●). Traces illustrate the time-courses of potential response; arrows (numbered) show the time of odorant application, and the following arrow (no number) shows the time of odorant removal. The number indicates the concentration of odorant applied in mol l⁻¹. Graph shows relative electrical resistance (R/R_0) as a function of concentration of the odorants, where R_0 stands for the resistance of the surface membrane of the droplet in the resting state. Temperature, 20 ± 1°C.

After the membrane potential had reached a steady value of about -90 mV, at which the droplet became electrically excitable, various concentrations of odorants were applied to the external medium. Intracellular potentials were recorded using a microelectrode. This resulted in a depolarisation of the membrane potential, recorded using a microelectrode, when the concentration exceeded a certain value.

Figure 1a represents two examples of the depolarisation when various concentrations of 1-octanol and isoamylacetate were applied to the droplet. The potential varied continuously with time and approached a steady value. Deviation of the steady potential from the original level is referred to as the olfactory response (OR), and the resting potential could be recovered after removal of the odorants. The membrane resistance of the protoplasmic droplet decreases markedly during excitation, as in squid giant axons^{8,9}. In contrast to electrical excitation, the membrane resistance of the droplet remained practically constant when the droplet was depolarised in response to odorants. Figure 1b shows the relative values of the electric resistance (R/R_0) of the surface membrane as a function of the concentration of the odorants, where R_0 stands for the membrane resistance of the droplet in the resting state.

When the odorants were applied to the internodal cell, the membrane potential varied in a complicated manner. Typical examples of the potential response are shown in Fig. 2. The potential varied sharply around 60 mV when the odorant was applied, decreasing slightly within a few seconds. It changed again with time, passed through a maximum and approached a steady value, returning to its original level when the odorant was removed from the

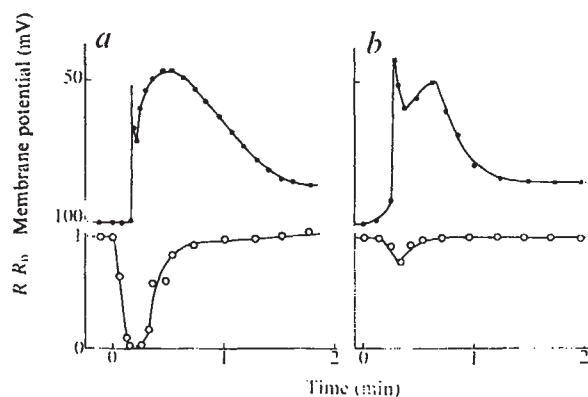
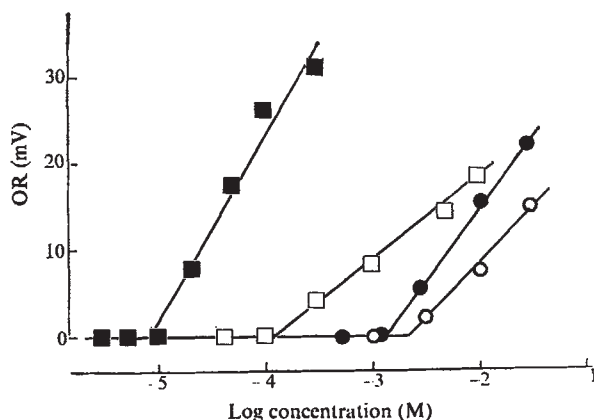


Fig. 2 Time-courses of variations in the membrane potential and the relative electrical resistance for an internodal cell in response to *a*, 3×10^{-3} M isoamylacetate and *b*, 5×10^{-4} M 1-octanol. Temperature, 23°C.

medium. The height of the first peak of the potential variation was independent of the odorant concentration, whereas that of the second peak and the final steady value of the membrane potential depended on the odorant concentration. Simultaneous measurements of the electrical resistance showed that the first peak of the membrane potential was accompanied by a marked decrease in the membrane resistance, as in the case of electrical excitation. This decrease recovered within 30 s, and the resistance was approximately constant during the following variation in the membrane potential (Fig. 2). The steady value for the electrical resistance of the membrane during olfactory response was almost the same as that in the resting state. When the internodal cell is not electrically excitable, no potential response is elicited after application of odorants.

Figure 3 shows the variations in the membrane potential at the steady value, OR, for the protoplasmic droplet, plotted as a function of odorant concentration (C) in the medium. The membrane potential did not change until the concentration of odorant reached a threshold value (C_{th}); OR increased almost linearly with increase in odorant concentration above C_{th} when OR was plotted against $\log C$. In the internodal cell, this linear relationship similarly held for the height of the second peak of the membrane potential change. The value of C_{th} determined from OR $\log C$ relationships for the protoplasmic droplet is in agreement with the olfactory threshold (T) for human¹⁰, and plots of $\log T$ against $\log C_{th}$ fall on a straight line of unit slope (Fig. 4). A similar unit slope relationship was obtained with the

Fig. 3 Deviation of the steady potential from the original level (OR) in response to odorants plotted against log odorant concentration (C) for the protoplasmic droplet. Experimental conditions as in Fig. 1. ■, 1-Octanol; □, isoamylacetate; ○, ethyl ether; ●, 1-butanol.



threshold observed in the internodal cell. The lack of variation in electrical resistance of the membrane both for the internodal cell and for the protoplasmic droplet during the olfactory response induced by 1-octanol or isoamylacetate seems to be difficult to interpret in terms of the puncturing theory¹¹, in which an odorant is assumed to puncture the olfactory receptor membrane temporarily, leading to a change in the ionic permeability of the membrane. The change in the membrane potential induced by odorants, at least in the above cases, may be attributed to a change in the potential produced at the membrane-solution interface as discussed in a recent study of taste reception^{12,13}. Further, the olfactory receptor of the box turtle responds to odorants

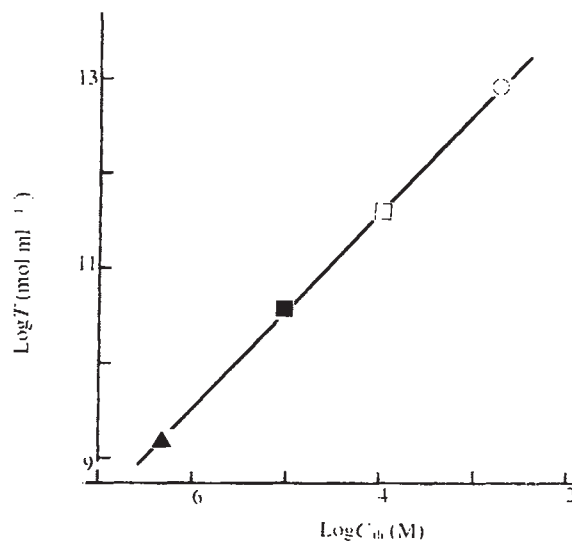


Fig. 4 Linear relationship with unit slope between $\log T$ and $\log C_{th}$, where C_{th} stands for the threshold of olfactory response for the protoplasmic droplet in *Nitella* and T represents the human olfactory threshold¹⁰. Symbols as in Fig. 3, plus skatol (▲).

even when a Na^+ -free solution is placed on the olfactory epithelium, and a removal of Ca^{2+} from the olfactory epithelium brings about an increase in spontaneous firing of the olfactory nerve¹⁴. The thresholds of the protoplasmic droplet to odorants were markedly lowered by a decrease in Ca^{2+} concentration in the medium. For example, the threshold for 1-octanol was lowered by a factor of 10^{-2} with a tenfold decrease in Ca^{2+} concentration. This decrease also increases the instability of the surface membrane of the droplet^{9,15}. It is therefore not unreasonable to consider that the extremely high sensitivity of the olfactory receptor cell to odorants may be attributed to the instability of the receptor membrane.

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Failure of exogenous gonadotrophin controlled ovulation to cause digit abnormalities in mice

GONADOTROPHINS have been used for many years in laboratory work to induce ovulation in animals at specific convenient times¹⁻⁵. An injection of pregnant mare serum (PMS) induces follicular growth, and if this is followed by an injection of human chorionic gonadotrophin (HCG) the mature follicles are ovulated at a predictable time. There are a few suggestions in the literature that this practice may lead to abnormalities in the offspring of the treated females⁶⁻⁸. In particular, Elbling⁸ reported that abnormalities in the digits and an altered sex ratio were found in litters born to gonadotrophin-treated female mice. As the practice of inducing ovulation by these means is widespread in research and is of practical importance in swine husbandry, we felt that this claim against PMS and HCG should be evaluated. We therefore repeated part of the experiment performed by Elbling⁸, with slight modifications, in that we did not study postnatal survival but observed digit normality, sex ratio and litter size in two stocks of mice in an effort to detect the influence that different genetic backgrounds may have in the response to exogenous gonadotrophins. We found no abnormalities in the digits or altered sex ratio in the offspring.

were sexed by making a mid-ventral incision and locating the gonads. Digits from all four paws were observed and if an abnormality was thought to be present, the paw was severed from the limb for study. It was then fixed, dehydrated, embedded in paraffin and sectioned with a rotary microtome at 8 μ m. Slides from the sample were stained with haematoxylin and eosin. The presence of all metacarpals and metatarsals was used as the criterion for digit normality together with a visual comparison made with digits from sections of the corresponding normal paw of the same or other animals.

Of the 640 offspring from 45 litters observed in the gonadotrophin-injected group, none showed missing digits on any limb. One foetus displayed an abnormal right hind limb which appeared to be lagging behind in development although all digits were found to be present on histological examination. In the control group of 266 foetuses from 24 litters, no digit or limb abnormalities were noted. Neither stock of mice, ICR or J, when compared with the controls for that stock by a χ^2 test, showed a significant difference in sex ratios. There was a significant increase in litter size in both gonadotrophin-treated stocks. This was not unexpected, however, and may be a result of superovulation caused by the gonadotrophin injections.

Both treated stocks showed an increase in prenatal mortality when compared with the controls. The ICR treatment group averaged 2.5 degenerations per litter compared with an average of 0.125 in the ICR controls. All of the ICR losses were judged to be early embryos because they were quite small and were being resorbed. The J treatment group showed an average of 0.54 prenatal deaths compared with 0.375 in the J controls. Of a total of 18 J treatment prenatal deaths, only five were late foetal deaths with the remaining 13 classified as resorbing early embryos.

Early embryonic mortality has been previously attributed to PMS-HCG controlled ovulation in mice by McLaren and

Table 1 Summary of data on reproduction of gonadotrophin-programmed mice

Stock	Treatment		Control	
	ICR	J	ICR	J
No. of litters	12	33	8	16
Total no. of foetuses	188	452	91	175
Average litter size*	15.67 $\pm$ 2.77†	13.7 $\pm$ 0.9†	11.38 $\pm$ 1.48	10.94 $\pm$ 0.82
No. of degenerating foetuses	30	18	3	2
Sex ratio*				
Male (%)	56.9	52.9	59.3	49.1
Female (%)	43.1	47.1	40.7	50.9

*Includes late degenerating foetuses but not degenerating embryos.

†Significantly different from control ($P < 0.05$).

Two random bred stocks of mice, ICR and J, were obtained from a colony maintained at Purdue University. The J stock was developed from four-way crosses between inbred lines LP/J, SJL/J, BALB/cJ, and C57BL/6J. The ICR mice originated from stocks with large litter size developed at the Institute of Cancer Research, Philadelphia, Pennsylvania. All female mice had borne one litter through natural mating and none had previously been exposed to any type of drug. Gonadotrophins were dissolved in 0.85% sterile NaCl solution such that each injection of 0.25 ml delivered 5 IU of PMS (NIAMDD, Bethesda, Maryland) or HCG (Ayerst, Chicago, Illinois).

Forty-five female mice, 12 ICR and 33 J were programmed to ovulate by intraperitoneal gonadotrophin injections of 5 IU PMS followed 48 h later by 5 IU HCG. Immediately after the second injection, each female was caged overnight with a male from the same stock. Twenty-four female mice, 8 ICR and 16 J, were used as controls. The presence of a copulatory plug (CP) the next morning after mating was used to determine day one of pregnancy. Seventeen days post-CP, the females were killed by cervical dislocation and their pups removed by dissection.

Litter size was determined and degenerating foetuses recorded. Average litter sizes include late foetal deaths but not early degenerating embryos. Using a stereomicroscope, pups

Michie⁹. In their study, using mice from Theiler's Original strain, treated adults had a higher incidence of embryonic mortality from 7.5 to 9 d of development than either treated immature females or controls. The specific mechanisms involved are not clear but several hypotheses have been forwarded⁹. These include maternal hormonal deficiencies, and delays in fertilisation because of improper timing of ovulation in relation to insemination so that 'aged' ova are fertilised. Delays in fertilisation have been shown by Butcher and Fugo¹⁰ to result in chromosome anomalies in rat embryos.

We found no evidence to link gonadotrophin-induced ovulation with digit abnormalities of the offspring, nor was any alteration of the sex ratio observed. This is in disagreement with Elbling⁸ who observed digit abnormalities in 5 out of 32 treated litters and a shift in the sex ratio in favour of females in litters of treated Swiss albino virgin mice.

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Cytochalasin B inhibits stabilisation of adhesions in fast-aggregating cell systems

THE rapid, naturally occurring aggregation shown by limpet haemocytes¹ and mammalian blood platelets^{2,3} is accompanied by production of prominent microspikes^{1,3} and is reversibly inhibited by cytochalasin B (CB)^{4,5}, thus differing from the slower aggregation of dissociated tissue cells⁶⁻⁸. Although CB inhibits aggregation of limpet haemocytes in shaken suspension, preformed aggregates shaken in the presence of CB are not disrupted, suggesting that CB inhibits the formation of stable contacts⁵.

By light microscopy, the first observable effect of CB on limpet haemocytes is collapse of the microspikes, apparently as a result of disruption of their supporting cores. An electron microscope study of the system (our unpublished work) confirms this by showing that CB causes rapid disappearance of the microfilament bundles (Figs 1 and 2) which constitute the core in these, as in other, microspikes⁹. The extreme rapidity and the reversibility of these two effects of CB, both the disappearance of microspikes and the inhibition of aggregation, suggests that these two phenomena may be causally linked. A possible basis for such a link is the theoretical requirement for low diameter projections to establish initial contact between similarly charged cell surfaces^{10,11}. The observation that haemocytes in the presence of CB spread on to a glass surface at a much slower rate than normal⁵, leads to the alternative suggestion that the contacts between colliding cells in shaken suspension are normally stabilised by the rapid spreading of participant cells over each other's surfaces by a microspike-dependent process similar to that by which they spread on to glass, and thus that spreading of microspikeless cells in the presence of CB is too slow to effect this stabilisation. We have attempted to distinguish between these hypotheses by studying the aggregation of limpet haemocytes maintained in prolonged contact in a centrifugal pellet in the presence of CB.

Cells in blood taken from the pallial vein of each of six limpets were submitted to the schedule shown in Fig. 3 as far as counts 3A-D, and in three of these cases, incubation in EDTA-seawater⁵ and counts 4A-D were added to estimate the number of cells adhering to the test tube. Particle numbers (one particle is one cell or a group of contacting cells) were estimated by haemocytometry and expressed as number per tube.

Essentially similar results were obtained from all the experiments, although there was considerable variation between cells taken from individual animals with regard to initial particle density, extent of aggregation and the number of cells lost by attachment to the test tubes. The results of a representative experiment are shown in Table 1. As expected from previous studies^{1,5}, haemocytes pelleted in artificial seawater (tube A) aggregate extensively within 5 min and, following dispersal, show no further aggregation on the shaker, presumably having reached an endpoint. In contrast, the preparations in CB disperse to give high particle numbers after incubation in the pellet for 5 min. With increasing incubation time, the dispersed particle number decreases progressively and by 30 min approaches the

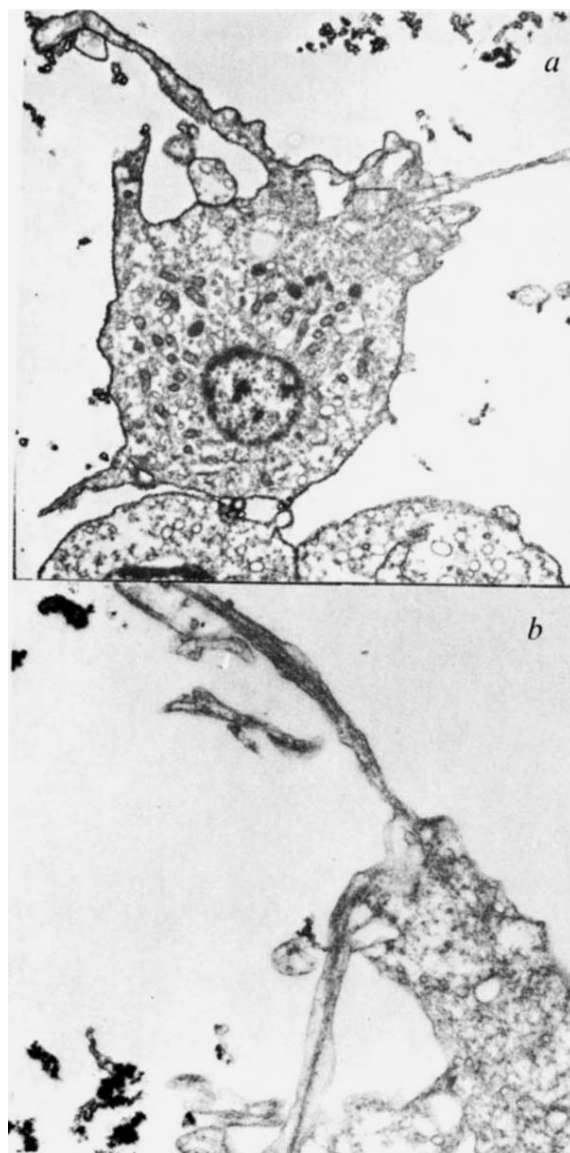


Fig. 1 *a*, Limpet haemocyte fixed from artificial seawater. Note well developed microspikes. ($\times 8,600$). *b*, High power of cell microspikes showing supporting core of microfilament bundles. ($\times 21,500$).

values seen in the artificial seawater control, with large aggregates appearing in the suspensions in the counting chambers. Counts 3B-D confirm that these aggregates are stable and also that no further aggregation occurs in the shaker system. It is evident from counts 4A-D that a negligible proportion of the particles is lost in the form of cells remaining attached to the glass after dispersal of the pellet. Thus it seems that cells maintained in contact in the presence of CB form stable adhesions to one another, but do so more slowly than do cells in artificial seawater.

The demonstration that stable contacts can be formed between CB-treated haemocytes does not preclude the hypothesis that spikes are normally required for the initiation of contact between cells in shaken suspension, for it is possible that the barrier of electrostatic repulsion between microspikeless CB-treated cells is overcome by the pressure of centrifugation. This hypothesis fails, however, to account for the fact that the degree of aggregation increases with incubation time. This observation suggests that haemocytes, in the absence of

Table 1 Effect of cytochalasin B on aggregation of pelleted limpet haemocytes

COUNT 1		6.6 ± 0.3 × 10 ⁵			
Initial particle no. per tube					
Tube					
Incubation medium	1 mm ³ cm ⁻³ ^A DMSO in ASW	^B	0.5 µg cm ⁻³ ^C CB in ASW	^D	
Incubation times in pellet	5 min	5 min	10 min	30 min	
COUNT 2					
Particle no. after dispersal of pellet	5.7 ± 0.84 × 10 ⁴	1.93 ± 0.13 × 10 ⁵	1.68 ± 0.16 × 10 ⁵	1.02 ± 0.07 × 10 ⁵	
Particle loss in pellet (%)	91	71	75	85	
P	0.001	0.001	0.001	0.001	
COUNT 3					
Particle no. after 5 min on shaker	4.7 ± 0.75 × 10 ⁴	1.82 ± 0.15 × 10 ⁵	1.64 ± 0.2 × 10 ⁵	1.12 ± 0.15 × 10 ⁵	
Particle loss on shaker (%)	17.5	5.7	2.4	-9.8	
P	0.4	0.6	0.9	0.8	0.5
COUNT 4					
No. of cells lost by adherence to test tube	3 ± 1.5 × 10 ³	4 ± 1.0 × 10 ³	6.5 ± 1.5 × 10 ³	4 ± 1.3 × 10 ³	

Results of a representative experiment following schedule shown in Fig. 3. Counts of particle numbers are expressed per tube. Percentage loss between two preceding counts are shown and the *P* values are calculated from *t* tests on the two preceding counts in each tube. ASW, Artificial seawater.



Fig. 2 Limpet haemocyte fixed after 20-s exposure to artificial seawater containing $0.5 \mu\text{g cm}^{-3}$ CB. Note total absence of microspikes. ($\times 12,900$).

microspikes, slowly increase the adhesiveness of their contacts, perhaps by spreading over each other in the same way that they spread slowly on to glass in the presence of CB⁶; a mechanism which would not be effective in a shaken suspension. The inhibition of rapid aggregation of haemocytes by CB can thus be accounted for entirely by the hypothesis that this fast aggregation depends on the stabilisation of adhesions by rapid spreading of frontal lamellae, in which the CB-sensitive microspikes play an important role (ref. 5 and our unpublished work). It seems possible that other rapidly aggregating cell systems such as blood platelets², thrombocytes¹² and aggre-

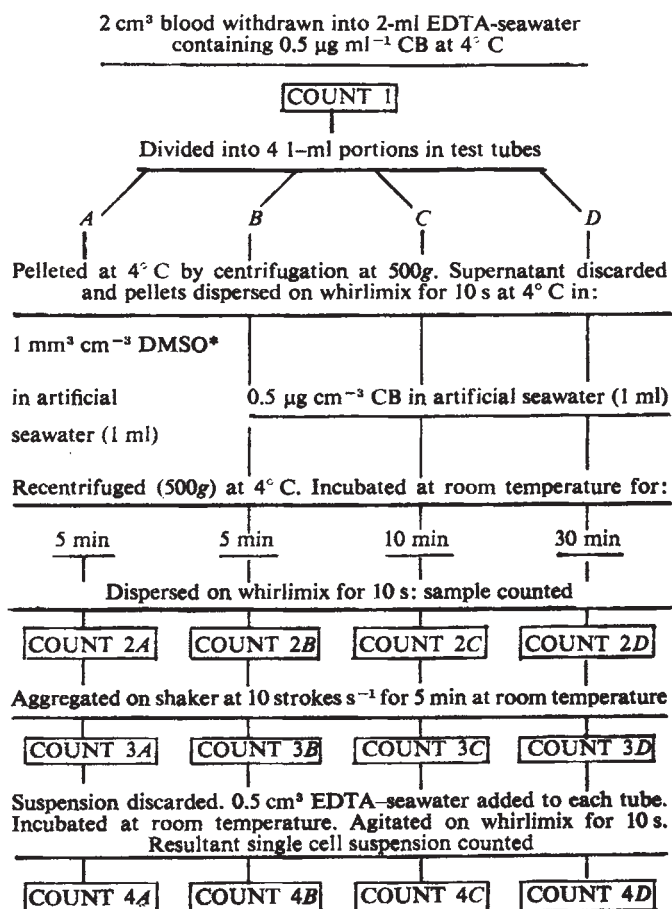


Fig. 3 Diagrammatic representation of experimental procedure used.

*DMSO was used in control experiments because stock CB solution was made up in this solvent.

gating slime mould cells¹³ may operate by a similar mechanism.

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Cell recognition by mucus secreted by urn cell of *Sipunculus nudus*

RECOGNITION of foreign particles in invertebrates is shown by the sticking of these particles to the circulating free amoebocytes of the host. The marine invertebrate *Sipunculus nudus*, phylum Sipunculida, has among its coelomic fluid ('blood') cells a remarkable 'urn cell' which secretes mucus that is sticky for foreign cells, but not for autologous cells. We have studied the biological specificity of this phenomenon and have attempted to alter the surface of autologous erythrocytes to render them sufficiently 'foreign' to stick to urn mucus.

Urn cells are bicellular organelles¹ about 20 µm in diameter, comprising a vesicular anterior cell and a loosely attached ciliated mucus-secretory cell (Fig. 1). The mucociliated cell originates in the epithelial lining of the coelom, detaches and becomes free-swimming in the fluid². Normally, as thousands

of urns swim about in the fluid their small sticky tails remove foreign and cellular debris from the coelom. If pathogenic bacteria or toxic materials are introduced into the coelom, however, urns are stimulated to produce long tails of mucus in which the invading substances are trapped. The host's own 'blood' cells (erythrocytes, non-activated amoebocytes, other white cells), never stick to either normal or actively hypersecreting mucus. Activated amoebocytes stick to both types.

Urn cells can be cultivated *in vitro* in sterile preparations of their own coelomic fluid. One drop of this fluid contains 50-100 swimming urns; their secretory activity can be observed directly in a depression slide by means of a dissecting microscope. The hypersecretory response can be provoked *in vitro* by the stimuli which induce it *in vivo*. Using this *in vitro* system, we had found³ that if the serum of acutely bacteraemic *S. nudus* was filtered and heated to 85°C for 5 min, one drop of this cell-free serum would induce rapid hypersecretion in all urns (Fig. 2). Unheated filtered serum had no effect. The molecular weight of the active molecule in the heated serum was found to be greater than 50,000 (ref. 4).

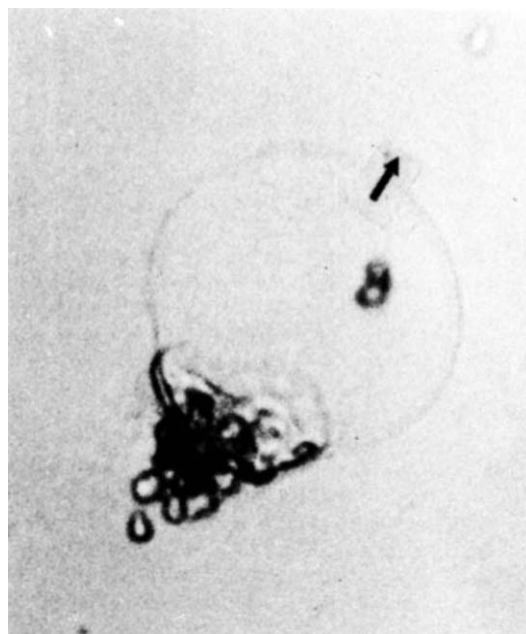
We studied, in the *in vitro* system, the biological specificity of the sticking of foreign cells to urn mucus. When heavy suspensions of *S. nudus* erythrocytes which had been washed three times were added to preparations of either normal or hypersecreting urns, none stuck to the mucus even though urns swam through dense fields of the cells. Table 1 shows that erythrocytes of two geographically isolated (Morgat, Locquémeau) colonies of *S. nudus*, and from three other species of Sipunculida also failed to stick. Red cells from five unrelated invertebrate species, however, were quickly trapped in both normal and hypersecretory mucus. Human and sheep red blood cells stuck immediately and avidly to normal mucus but much more slowly and less densely to hypersecreted mucus. Sperm from three of the invertebrates were immediately and thickly trapped in hypersecreting mucus; normal secretion has not yet been tested. White blood cells from *Ascidia mentulis*, the crab *Carcinus maenas* and the sea star *Asterias rubens* (the latter two withdrawn into 0.01 M N-ethylmaleimide (NEM) to prevent agglutination) were trapped densely by normal, and somewhat less so by hypersecreting, mucus. Live *Anophrys*, a ciliated protozoan which is tolerated by some invertebrates but is lethal to others, was not trapped; but when inactivated by NEM it was promptly trapped.

Table 1 Degree of adherence of autologous and foreign cells to urn cell mucus

		Degree of adherence to mucus	
Species	Cell type	Normal mucus tails	Hypersecretory tails
<i>S. nudus</i> colonies			
Locquémeau area	RBC	0	0
Morgat area	RBC	0	0
Related species of Sipunculida			
<i>Phascolosoma vulgare</i>	RBC	0	0
<i>P. elongatum</i>	RBC	0	0
<i>Phascolion strombi</i>	RBC	0	0
Unrelated species			
<i>Arenicola</i>	RBC	+++	+++
<i>Glycera</i>	RBC	+++	+++
<i>Notomastus</i>	RBC	+++	+++
<i>Nereis</i>	RBC	+++	+++
<i>Ascidia</i>	RBC	+++	+++
Sheep	RBC	+++	+
Human	RBC	+++	+
<i>Asterias</i>	Sperm		+++
<i>Ascidia</i>	Sperm		+++
<i>Notomastus</i>	Sperm		+++ +
<i>Ascidia</i>	WBC	+++	++
<i>Carcinus</i>	WBC*	+++	++
<i>Asterius</i>	WBC*	+++	++
<i>Anophrys</i> (living)	Protozoan	0	0
<i>Anophrys</i> (inactivated)	Protozoan*		+++

* Withdrawn into 0.01M into N-ethylmaleimide (NEM).
Blanks indicate no test.

Fig. 1 Urn cell with normal mucous tail.



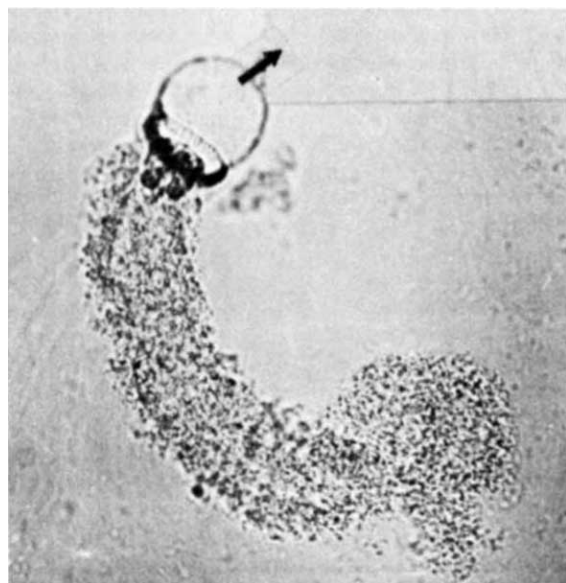


Fig. 2 Hypersecreting urn cell.

We then studied the response of hypersecretory mucus to autologous erythrocytes which were treated in various ways to alter the cell membrane. As Table 2 shows, if red cells were heated to 60° C for 5 min there was moderate sticking, if heated to 85° C for 5 min the heat-killed cells adhered thickly (Fig. 3). If the heat-killed cells were washed in, and resuspended in, a seawater suspension of 1 mM EDTA, they failed to stick. Red blood cells were incubated with five enzymes known to contain powerful proteases and/or glycosidases; with a marine vibrio presumed to contain sialidase (many strains do); and with concanavalin A with the thought that it might bind with cell surface sugars⁵ and mask these sites. Treatment with clarase, which contains α amylase, resulted in sticking of some of the erythrocytes to the mucus, but none of the tested enzymes was as effective as heat-killing.

Roseman⁶ has proposed that cell adhesion may involve an enzyme-substrate interaction between glycosyl transferases and complex carbohydrates. Cell-free urn mucus offers a natural substrate for studies of cell adhesion. Urn cell mucus can apparently recognise (trap) foreign cells from both vertebrate and invertebrate hosts other than sipunculids; whether the mucus or the cell is the determining substrate is an open question. The fact that human and sheep cells are trapped with

differential avidity by normal and by hypersecreted mucus suggests structural differences in the two types of mucus.

It should now be possible to determine (1) what specific chemicals will interfere with the recognition of foreign cells by mucus, and (2) which enzymes will change the host cell surface so that it is recognised as foreign by mucus.

We thank Dr J. Bergerard of the University of Paris, Director of the Station Biologique de Roscoff, and the administrative staff of the station for all facilities; Dr J. Vasserot for inverte-

Table 2 Treatment of *S. nudus* red blood cells in attempts to alter cell membrane and achieve 'foreignness,' as measured by sticking of RBC to hypersecreted urn mucus

Substance used	Time of incubation with RBC	Temperature (°C)	Degree of sticking to mucus
<i>S. nudus</i> RBC	(Unheated)	20	0
<i>S. nudus</i> RBC	Heated 5 min	60	++
<i>S. nudus</i> RBC	Heated 5 min	85	+++
Marine vibrio	30 min	20	0
Rhozyme 1%	30 min	20	0
Bromelain 1%	30 min	30	0
Clarase 1%	30 min	20	0
Hyaluronidase 1%	30 min	20	0
Pronase 1%	30 min	20	+
Concanavalin A 1%	30 min	20	0

Rhozyme HP-150 (Rohm and Haas), from *Aspergillus niger*, contains many glycosidases plus some proteases. Pineapple bromelain (Dole, Hawaii) is a powerful proteolytic enzyme, but also contains glycosidases. Clarase (Miles), from *Aspergillus oryzae*, contains α -amylase and many other glycosidases.

brate species other than *S. nudus*, and Dr Y. C. Lee of the Department of Biology, Johns Hopkins University, for advice and counsel, as well as for the test enzymes.

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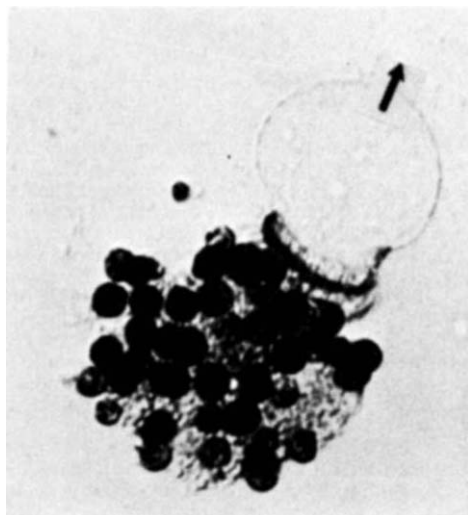
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Fig. 3 Hypersecreting urn cell with adherent heat-killed erythrocytes.



Glucose-induced decrease in Rb⁺ permeability in pancreatic β cells

THE membrane potential of pancreatic islet cells varies with the extracellular D-glucose concentration¹, from about -33 mV at zero glucose to about -16 mV at 28 mM, and 3-17 mM D-glucose induces bursts of rhythmic depolarisations with an amplitude of a few mV. Similar observations have been obtained², but with a membrane potential of about -60 mV in the absence of glucose. Comparisons of various sugars revealed a qualitative correlation between depolarising and insulin-releasing capacities¹; continuous recordings from single islet cells revealed a quantitative correlation between spike frequency and D-glucose concentration^{2,3}. Depolarisation of the β -cell membrane may therefore be essential for the triggering of insulin secretion¹⁻³. We report some experiments which indicate that the diminishing effect of D-glucose on membrane potential may be caused by a decrease in K⁺ permeability.

The ionic basis of membrane potential and rhythmic depolarisations in the pancreatic β cell is largely unknown,

although there are indications that the latter may predominantly reflect Ca^{2+} influx⁴. Using $^{86}\text{Rb}^+$ as a functional analogue of K^+ , we suggested⁵ that a Na^+/K^+ -pump in the β cell is capable of concentrating Rb^+ more than fortyfold from 70 μM RbCl in the extracellular fluid⁵. Measurements of $^{22}\text{Na}^+$ uptake indicated that β cells in equilibrium with a physiological buffer contain as much as 95 mM Na^+ inside⁶. Thus, the relatively small membrane potential in the β cell may tentatively be attributed to a comparatively great permeability to Na^+ , a low Na^+/K^+ -pump activity, or both. Since D-glucose was not found to alter the fluxes of Na^+ (refs 5 and 6), however, the glucose-induced decrease in membrane potential is not readily explained in terms of a further increase in Na^+ permeability or decrease in pump activity.

Fresh pancreatic islets (containing more than 90% β cells), were microdissected free-hand from adult non-inbred *ob/ob* mice and incubated for 2 h at 37°C in Krebs-Ringer bicarbonate buffer equilibrated with $\text{O}_2:\text{CO}_2$ (95:5) and containing (mM): 143.5 Na^+ , 5.9 K^+ , 2.6 Ca^{2+} , 1.2 Mg^{2+} , 128.3 Cl^- , 25.0 HCO_3^- , 1.2 SO_4^{2-} , 1.2 H_2PO_4^- , 3.0 D-glucose, 0.07 $^{86}\text{Rb}^+$ (290 Ci mol^{-1}) and 0.1 6,6'- ^3H -sucrose (150 Ci mol^{-1}). The same type of medium without Rb^+ and sucrose was used as basal medium in the following. After brief rinsing (~2 s) in basal medium, incubation was continued in basal medium only, or in basal medium supplemented with 17 mM D-glucose, L-glucose or 3-*o*-methyl-D-glucose. The islets were then freeze-dried overnight (-40°C, 0.1 Pa), weighed on a quartz-fibre balance and analysed for radioactivity in a liquid-scintillation spectrometer. Because sucrose is distributed as an extracellular space marker in microdissected islets⁷, the Rb^+ content was corrected for ^{86}Rb in the sucrose space; however, this correction was a minor one, as the sucrose space contained less than 5% of the total Rb^+ content.

Table 1 Content of $^{86}\text{Rb}^+$ in islets preloaded with the isotope and subsequently incubated for 10 min in non-radioactive media of different sugar composition

Sugar in medium	No. of experiments	$^{86}\text{Rb}^+$ (nmol) retained per kg dry weight of islets
D-glucose (3 mM)	18	1.53 ± 0.15
D-glucose (20 mM)	18	1.96 ± 0.10
D-glucose (3 mM) plus L-glucose (17 mM)	17	1.53 ± 0.09
D-glucose (3 mM) plus 3- <i>o</i> -methyl-D-glucose (17 mM)	18	1.57 ± 0.07

Mean values $\pm$ s.e. Control islets (3 mM D-glucose) released about 40% of their Rb^+ content in 10 min; to express results in mM, the tabulated values should be divided by 1.2, as the intracellular water of incubated islets amounts to about 1.2 l per kg dry weight (refs 5 and 7).

Table 1 shows the amount of Rb^+ retained by the islets during a subsequent 10 min incubation in non-radioactive medium. The islets retained significantly more Rb^+ when the extracellular D-glucose concentration was 20 mM than when it was 3 mM ($P < 0.025$). This effect of D-glucose could not be reproduced using L-glucose or 3-*o*-methyl-D-glucose. Since the disappearance of Rb^+ from preloaded islets seems to be an exponential process that probably reflects unidirectional efflux of the ion⁶, the inhibitory action of D-glucose is most simply interpreted as a decrease in Rb^+ permeability. Moreover, as Rb^+ can effectively substitute for K^+ in the Na^+/K^+ -pump and D-glucose had no demonstrable effect on Na^+ fluxes^{5,6}, it seems reasonable to assume that our Rb^+ data reflect properties of the system governing passive K^+ fluxes in the β cell.

The clear specificity of the inhibitory effect of D-glucose is interesting in view of the fact that, among the sugars tested here, only D-glucose is capable of stimulating insulin secretion and of inducing electrical activity in the islet cells¹. According to the generally accepted theory of the genesis of neuro-membrane potential, the decisive factors are an active estab-

lishment of opposing chemical gradients of Na^+ and K^+ in conjunction with a greater passive permeability to K^+ than to Na^+ . There are striking similarities between neurones and β cells in that both exhibit a negative membrane potential, have a Na^+/K^+ -pump, and are depolarised by high concentrations of extracellular K^+ (ref. 4). We therefore suggest that the depolarising action of D-glucose on pancreatic β cells may be mediated, at least in part, by a decrease in K^+ permeability.

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Macrophage proliferation *in vitro* induced by exudates

MOUSE peritoneal macrophages in culture are blocked in the G_0 phase of the cell cycle¹ and can be stimulated to synthesise DNA and divide *in vitro* only by exposure to feeder layers or to medium conditioned by other cell types²⁻⁴. A similar system has been reported for mouse alveolar macrophages⁵. This reluctance to divide *in vitro* contrasts with the striking proliferation of inflammatory macrophages *in vivo*^{6,7}.

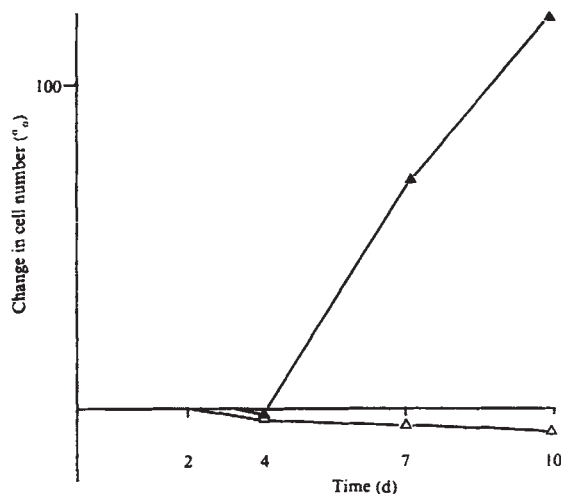


Fig. 1 Increase in cell numbers shown by counts over the same area of coverslip using an inverted microscope at sequential intervals during the culture period. Exudate was added at time zero. Cell cultures unstimulated peritoneal cells were obtained by lavage in medium 199 from young adult male Porton mice, were seeded on to 6 mm glass cover slips in Falcon Microtest II plates; settled for 2 h at 37°C, washed vigorously to remove non-adherent cells and transferred to Sterilin LM plates sealed with a 19 mm glass coverslip. Cell-free exudate was raised in the same donor mice by the subcutaneous implantation of modified diffusion chambers. These consisted of 20 mm lengths of nontoxic polystyrene tubing (10 mm diameter) sealed at each end by a Millipore membrane filter (pore size 0.45 μm) and filled with Medium 199. Four days after implantation, protein-rich exudates were recovered from the chambers, filtered through Millipore membrane filters (0.45 μm) and applied directly to the cell cultures. Culture media were medium 199 (penicillin 100 U ml^{-1} and streptomycin 100 μg ml^{-1}) and foetal bovine serum. Test cultures (▲) received 50% exudate, 25% foetal bovine serum and 25% medium 199. Control cultures (Δ) received 75% foetal bovine serum and 25% medium 199.

We have now been able to initiate *in vitro* proliferation in cultures of mouse peritoneal macrophages by the addition of inflammatory exudates. Such cultures exhibited extensive nuclear incorporation of tritiated thymidine, a high incidence of mitotic figures and a considerable increase in total cell numbers as demonstrated by direct counting (see Fig. 1) compared with control cultures not exposed to exudate.

Macrophage proliferation was seldom demonstrable earlier than four days after addition of exudate to the culture. Phagocytosis of human red blood cells and heat-killed *M. tuberculosis*, and histochemically evident acid phosphates, seemed higher in cultures treated with exudate than in the controls. Electron microscopy revealed features typical of macrophages, and the cells treated with exudate seemed to have more cytoplasmic organelles and fimbriae than control cells. Preliminary characterisation of the mitogenic factor present in the exudate has so far indicated only that it is relatively thermostable, being unaffected by freezing and thawing, storage for 6 months at -25°C or by incubation for 1 h at 65°C . It is not yet possible to correct it with the macrophage-derived factors which affect mitosis in other cell types⁸.

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Induction of DNA synthesis in rat macrophages *in vitro* by inflammatory exudate

The proliferation of macrophages in inflammatory exudates¹⁻³ contrasts with the failure of these cells to divide *in vitro* except in the presence of highly specialised conditioned media⁴⁻⁷. Macrophage division *in vivo*, however, could be explained if inflammation produced a local mitogenic factor.

Inflammatory exudates were produced in DFA-specified pathogen-free rats by intrapleural injection of 1 ml 6% Dextran (40,000 daltons) and collected 4 h later. The exudate was centrifuged and the supernatant passed through a Millipore filter (pore size 0.22 μm). The filtered exudate was diluted with medium 199 plus 20% newborn calf serum to make a final concentration of exudate of 30 or 50%.

Macrophages were collected from DFA rats by washing the peritoneum with medium 199. Macrophages were also collected 4 d after intraperitoneal injection of nutrient broth (activated macrophages). The macrophages were then cultured in Leighton tubes⁸ and 3 d later the medium was replaced with medium containing exudate. Three to six days after the addition of exudate DNA synthesis was assessed by autoradiography after incorporation of a single 30 min pulse of tritiated thymidine. The results of a typical series of experiments (Fig. 1) show that both normal and activated macrophages responded after 5 d of exposure to exudate *in vitro*. The labelling index of the treated cells rose to 20–30% compared with virtually zero in the control macrophages. Samples taken earlier than 5 d showed much less labelling. The macrophages synthesising DNA showed avid phagocytosis of starch particles added to the medium, which seemed to be greater than in the controls. Preliminary experiments have shown that exudates from Lewis rats are active on peritoneal macrophages from inbred Lewis rats and also on mouse peritoneal macrophages.

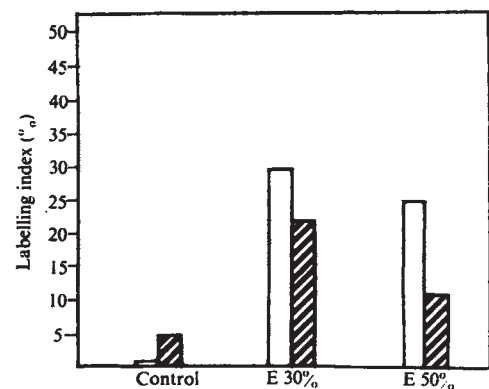


Fig. 1 Uptake of tritiated thymidine by 'normal' and 'activated' macrophages 5 d after culturing with or without exudate added to the culture medium. The open blocks represent normal and the hatched blocks activated macrophages.

The chemical nature of this stimulatory factor is unknown and the possibility of it being a virus cannot be excluded. It does not seem, however, to result from treatment with dextran alone, as dextran added to culture medium and passed through Millipore filters failed to induce DNA synthesis in macrophages. Its relationship to the factors released from peritoneal macrophages which stimulate or inhibit other cell lines remains to be determined⁹.

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Effect of antibody emission rates on plaque morphology

CUNNINGHAM and Fordham¹ have used a modified version of the haemolytic plaque technique to show that antibody diversity can be generated after B cells have been stimulated to proliferate. They tested antibody plaque forming cells (PFC) against a mixture of sheep red blood cells (SRBC) from two different sheep and obtained three morphological classes of direct plaques: clear (the plaque radii for both types of SRBC were equal), sombrero (the plaque radii were different for the two types of SRBC), and partial (there was only one plaque radius). For the clear and sombrero plaques both types of SRBC lysed to some extent, while for the partial plaques only one type of SRBC lysed. Cunningham and Fordham used plaque morphology as an antibody specificity marker, that is, if two plaques were different in morphology this was taken to mean that the antibodies produced by the two PFC differed in their specificity for the two types of SRBC. They studied clones produced from single PFC and found in most cases that the plaques produced by the progeny were of the same morphological class, differing only by slight variations in their plaque radii. In 10 of the 93 clones observed, however, the morphology of the plaques differed within the clone. In 7 clones two different morphological classes of plaque were observed, and in the others all three classes. Cunningham and Fordham suggested that within a given clone the antibodies emitted by different cells can have different specificities.

We report here that changes in plaque morphology do not

Table 1 Plaque radii

S (antibodies s^{-1})	r_1 (mm)	r_2 (mm)	A_1/A_2
10^2	0.59	0.02	870
10^3	0.76	0.15	36
10^4	0.94	0.28	11
10^5	1.10	0.45	6

S , antibody (Ab) emission rate; r_1 , distance from the PFC to the edge of lysis of type 1 SRBC; r_2 , distance to the edge of lysis of type 2 SRBC. The last column lists the ratio of the areas of lysis. These radii were calculated using equations (5) and (6) and the values of the parameters listed below Fig. 1.

necessarily indicate changes in antibody specificity, but can be caused by changes in the rate at which antibodies are emitted from the PFC, that is, two plaques formed by antibodies with identical specificities can be of different morphological classes if the antibody emission rates of the PFC differ.

If we consider lymphocytes which emit antibodies with identical specificities that form multivalent attachments to epitopes on the surface of type 1 SRBC and monovalent attachments to epitopes on the surface of type 2 SRBC, then at low antibody emission rates the concentration of bound antibodies on type 2 SRBC may be too low to produce observable lysis in the presence of complement so that a partial plaque is obtained. At high antibody emission rates the bound antibody concentration will be higher on both types of SRBC. If the concentration is high enough on the type 2 SRBC a sombrero will occur. I show here that such changes in plaque morphology can occur for reasonable values of the binding parameters and emission rates.

Mathematical descriptions of plaque formation due to single antibody emitting cells in thick gels containing SRBC have been presented^{2,3}. It is straightforward to modify the theory for a thin layer (diffusion only in a plane) containing two types of SRBC. For the case just discussed, in which the antibodies form multisite attachments to binding sites on type 1 SRBC (strong binding) and single site attachments to binding sites on type 2 SRBC (weak binding), equations (1)–(3) describe such binding where there is a lymphocyte at the origin emitting antibodies (IgM in our case) at a rate S , and where these antibodies diffuse into a layer containing a homogeneous mixture of the two types of SRBC.

$$\frac{\partial c}{\partial t} = D \nabla^2 c - \frac{1}{n} \frac{\partial \rho_1}{\partial t} + \frac{\partial \rho_2}{\partial t} + \frac{S}{h} \delta(r) \quad (1)$$

$$\frac{\partial \rho_1}{\partial t} = -k_{11} n c \rho_1 \quad (2)$$

$$\frac{\partial \rho_2}{\partial t} = -k_{21} c \rho_2 + k_{22} (\rho_{20} - \rho_2) \quad (3)$$

c is the concentration of free antibody, ρ_1 and ρ_2 the concentration of free binding sites on type 1 and 2 SRBC, h the layer thickness, k_{11} the antibody–SRBC1 forward rate constant, n the average number of bound sites per multisite attachment, k_{21} and k_{22} the antibody–SRBC2 forward and reverse rate constants, r the position vector (two-dimensional), $\delta(r)$ the two-dimensional Dirac delta function, D the diffusion coefficient of the antibody and ∇c the concentration gradient of antibody. At time $t = 0$, $c = 0$, $\rho_1 = \rho_{10}$ and $\rho_2 = \rho_{20}$. In equation (2) the change in the binding site concentration caused by detachment of antibodies bound multivalently is neglected. This is equivalent to assuming that once an antibody binds multivalently it remains bound until the end of the experiment (one hour or less in this case).

When the fraction of binding sites occupied is small these equations can be linearised and solved^{2,4}. (Note that the fraction bound can be small even though large numbers of antibodies are bound per RBC. This is because of the large number of sites per RBC, for example, 6×10^8 sites per SRBC for the Forssman epitope⁵.) For times longer than a few seconds local equilibrium will exist between the free and monovalently bound antibodies.

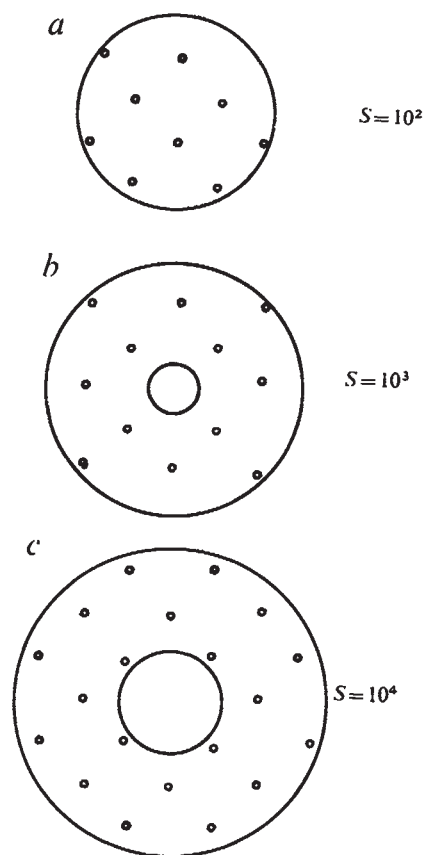


Fig. 1 Three plaques calculated from equations (5) and (6) for three values of the emission rate S . The values of the plaque radii are listed in Table 1. The clear area represents lysis of both type 1 and 2 SRBC. The area with small circles present represents lysis of only type 1 SRBC. Beyond the outer circle there is no lysis. Plaques with radii smaller than 0.05 mm are assumed to be non-observable. a , $S = 10^2$ antibodies s^{-1} . The plaque is partial. b , $S = 10^3$ antibodies s^{-1} . c , $S = 10^4$ antibodies s^{-1} . The plaques are sombrero. The values of the parameters used to calculate the plaque radii in Table 1 are as follows: $E_1 = 10^3$ sites per RBC, $E_2 = 5 \times 10^3$ sites per RBC, $K = 10^6 M^{-1}$, $1.66 \times 10^{-15} cm^3$ per antibody, $n k_{11} = 10^3 M^{-1} s^{-1}$, $1.66 \times 10^{-16} cm^3$ per antibody s^{-1} , $h = 2 \times 10^{-3} cm$, $D = 10^{-7} cm^2 s^{-1}$, $\rho = E_1 \rho_{0RBC}$ and $\rho_{RBC} = 2 \times 10^8 RBC cm^{-3}$, $n = 2$ and $t = 3,600 s$. The number of IgM molecules bound per SRBC at the plaque radius was taken to equal one. For a discussion of these parameters see ref. 3.

Further, for the values of the parameters we consider, $k_{11} \rho_{10} t \gg 1$, in which case the solutions to equations (1)–(3) become:

$$c(r, t) = (S/2\pi Dh) K_0(\lambda r) \quad (4)$$

$$N_{b1} = E_1 \{1 - \exp[-k_{11} n t c(r, t)]\} \quad (5)$$

$$N_{b2} = K E_2 c(r, t) \quad (6)$$

(In general $c(r, t)$ can be written as the sum of a time-independent term, equation (4), and a time-dependent term. When $k_{11} \rho_{10} t \gg 1$, the time-dependent term is negligible.) N_{b1} and N_{b2} are the number of bound antibodies per SRBC at position r and time t on type 1 and type 2 SRBC, E_1 and E_2 are the number of binding sites on type 1 and 2 SRBC at $t = 0$, $K = k_{21}/k_{22}$, K_0 is a modified Bessel function⁶ and $\lambda = (k_{11} \rho_{10}/D)^{1/2}$.

To obtain values for the plaque radii we must know the number of antibodies bound per SRBC at the edge of the plaque. For IgM this number has been estimated to be between one and ten³. We take it to equal one. When we set $N_{b1} = N_{b2} = 1$, equations (5) and (6) become equations for the plaque radii r_1 and r_2 , respectively. Using these equations we can predict how the plaque radii change as functions of the various parameters.

Figure 1 and Table 1 show how the plaque size and morphology vary as a function of antibody emission rate. The three plaques in Fig. 1 were obtained for $S = 10^2$, 10^3 and 10^4 anti-

bodies s^{-1} . (Experimental studies have yielded estimates of S in the range $0.5 \times 10^{-1} - 2 \times 10^4$ antibodies s^{-1} (ref. 7).) All the parameters except S have been held fixed, including the various binding constants. Thus the antibody specificity is the same for the three plaques in Fig. 1. By increasing S the plaques change from partial to sombrero.

We have shown that differences in the antibody emission rate of a clone member can cause it to produce a plaque which differs in morphology from those plaques produced by other clone members. We have demonstrated transitions between partial and sombrero plaques. One other question to consider is whether changes in the antibody emission rate can cause progeny of a clear PFC to yield other than a clear plaque. If the antibody binding constants are identical for types 1 and 2 SRBC, a clear plaque will be produced for all antibody emission rates. A clear plaque, however, does not necessarily mean the antibody has identical binding constants for both types of SRBC. It may instead mean that the differences in specificity are not sufficient to produce a measurable difference in the plaque radii. Whether a large change in the emission rate could lead to two well defined plaque radii (a sombrero) is an open question.

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DR CUNNINGHAM REPLIES—Plaque morphology has been used¹⁻⁵ as a simple way of distinguishing cells making different antibody specificities. Other interpretations of the meaning of plaque differences are possible, and Goldstein⁶ has suggested that rate of antibody secretion is decisive. As with any complex assay system, it is impossible to discount such alternative interpretations absolutely. The question is not, 'are such artefacts conceivable?'—for evidently they are—but 'are they likely to occur under the experimental conditions?' Control experiments and arguments for the validity of plaque morphology as a specificity marker have been presented elsewhere⁷. Some of these are listed below.

Goldstein's argument requires that plaques with different morphologies should have different overall (largest) diameters. In fact, in many of our clones, the outer diameter of partial lysis was similar in most or all plaques, while the clear area of total lysis varied from one cell to the next. In effect, overall plaque size acts as a control against differences in rate of antibody production per unit time. His argument could, however, be rescued with more complicated assumptions, for example rapid fluctuations in rate of antibody release.

To eliminate this we watched plaques develop. Those which started as sombreros (after incubation for 15 min) always grew into larger sombreros with the same relative areas of clear and partial lysis at 1 h (ref. 7). Clear and partial plaques, (the 'extreme cases' of sombrero morphology), behaved similarly.

Individual plaque-forming cells, micromanipulated from one red cell monolayer to another, produced plaques of similar morphology at different temperatures⁷. That is, rate of antibody release did not seem to affect plaque morphology.

Clones grown *in vivo*^{1,8} often contained several thousand antibody-forming cells all with exactly the same morphology on a mixed indicator monolayer. Others had varying proportions of two types, for example half were clear and half partial, with no intermediate forms. It is not an indisputable argument, but if plaque morphology depends critically on rate of antibody

release, one would expect to find a spectrum of types in any collection of plaques. This observation of very large numbers of homogeneous plaques also serves to discount the suggestion that variation in rate of production is a rare event which our control experiments failed to detect.

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Temperature-sensitive growth of cells transformed by *ts-a* mutant of polyoma virus

ONE of the characteristics of the transformed state in culture in mammalian fibroblasts transformed by polyoma virus and Simian Virus 40 (SV40) is the lowered serum requirement for cellular growth¹⁻⁴. The participation of a viral gene(s) in this attribute of transformation has been known for polyoma virus⁵ and SV40 (refs 6 and 7). Recent experiments show that some of the other properties associated with the transformed state are also under the control of the same viral gene(s)⁶⁻¹¹. The *ts-a* gene of polyoma virus controls the initiation of stable transformation in the hamster BHK 21 cell line¹², although it is usually accepted that the function of this gene is not required for the maintenance of the transformed state¹²⁻¹⁴. I report here the temperature-sensitive growth in low serum medium of some of the rat cell lines transformed by the *ts-a* mutant of polyoma virus, and suggest that this viral gene controls at least one aspect of the maintenance of transformed state in certain transformed cells.

Like other untransformed fibroblastic cells widely used in transformation experiments^{1-3,15}, 3Y1 rat cells require a high concentration of serum for growth and exhibit poor growth in medium containing low concentrations of serum, whereas 3Y1 cells transformed by polyoma virus or SV40 can grow well in low serum medium (Kimura and Kaneto, unpublished). To determine whether the *ts-a* gene of polyoma virus affects the growth properties of transformed cells, seven independent 3Y1 lines transformed by *ts-a* mutant at permissive temperature as well as three 3Y1 lines transformed by wild type (WT) virus were examined for their ability to grow in low (2%) and high (10%) serum medium at 33°C and 40°C, the permissive and non-permissive temperatures for the productive cycle of this mutant^{15,16}. Figure 1 shows that the growth of untransformed 3Y1 cells was reduced in 2% serum at both 33°C and 40°C, compared with that in 10% serum. Growth in 2% serum of three of the 3 WT-transformed lines (Py-3Y1-3, 4 and 117) was also reduced at both temperatures, compared with that in 10% serum, but it was still much better than that of 3Y1 in 2% serum at both temperatures. A striking difference in cellular growth was observed in 3Y1 lines transformed by *ts-a* mutant. There was much less growth in four out of the seven *ts-a*-3Y1 lines (*ts-a*-3Y1-1, 2, 3 and 6) in 2% serum at 40°C than at 33°C. Two lines (*ts-a*-3Y1-3 and 6) further showed less growth in 10% serum at 40°C than 33°C. Three *ts-a*-3Y1 lines (*ts-a*-3Y1-4, 5 and 7) showed little or no detectable temperature-sensitivity for growth under the conditions, thus resembling WT-transformed 3Y1 cells. Figure 2 shows the growth curves of one of the *ts-a*-transformed 3Y1 lines (*ts-a*-

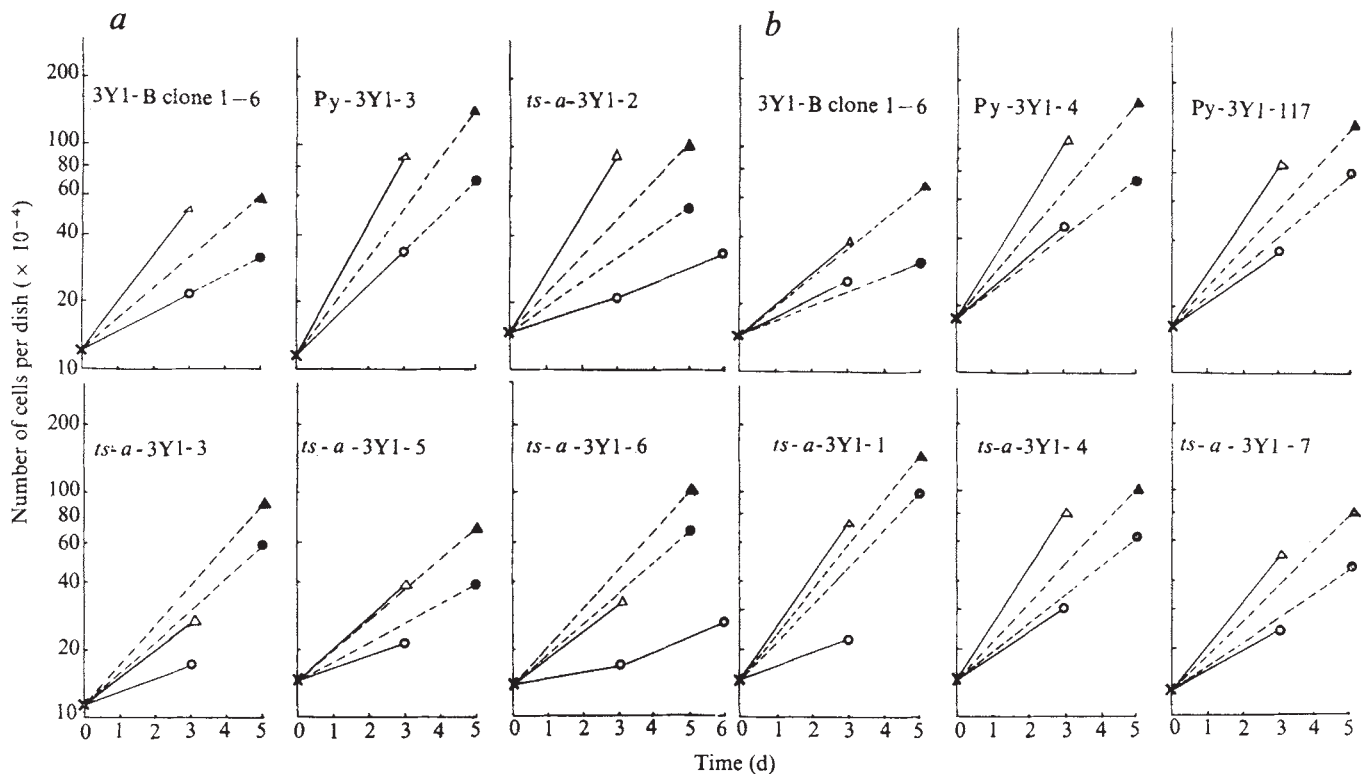


Fig. 1 *a* and *b*, Growth in different serum concentrations of 3Y1 cells at 40° C and 33° C after transformation by polyoma virus and by *ts-a* mutant of polyoma virus at permissive temperature. Cells grown at 33° C (transformed lines) and at 37° C (3Y1-B 1-6) in medium containing 10% foetal bovine serum were dispersed with a trypsin-Versene mixture, suspended in Vogt-Dulbecco medium containing 1.5% serum, counted in a haemocytometer and diluted in the same medium. The cell suspension (5 ml) was inoculated into each 50-mm plastic Petri dish (2×10^5 cells per dish). After incubation at 33° C for 3 h to allow cells to settle, the cultures were replaced with 5 ml of fresh medium containing 2% or 10% serum. Half of the cultures were placed in an incubator at 40° C, the other half at 33° C (zero time). At zero time and at indicated times of incubation, cells were trypsinised and counted. Accuracy of cell count is within 10%. *a* and *b* represent different experiments: ○, 2% serum at 40° C; △, 10% serum at 40° C; ●, 2% serum at 33° C; ▲, 10% serum at 33° C. A stock of WT polyoma virus (M. Vogt, clone LP147) was prepared in mouse kidney cultures. A stock of *ts-a* mutant of polyoma virus¹⁵ (Dulbecco) was prepared in BALB/3T3 cells. All transformed lines used were derived from a cloned population of the rat cell line 3Y1 (3Y1-B clone 1) (ref. 16, and G.K., A. Itagaki and J. Summers, unpublished). The 3Y1 cells were infected with WT or *ts-a* at an input multiplicity of about 50 and incubated either at 37° C (WT) or first at 33° C for 2 d and then at 37° C (*ts-a*). After 2–3 weeks, transformed colonies were identifiable as the relatively thick colonies composed of cells randomly oriented and piled up on each other (criss-cross) as described previously (G.K., A. Itagaki, and J. Summers, unpublished). Transformed cell lines were developed at 37° C by choosing well isolated colonies. Only one colony was selected from the given Petri dish, to obtain independent cell lines. To avoid possible secondary alterations, care was taken to prevent crowding. The cell lines were stored at –70° C at their early passages. The transformed cells were grown and passaged at 33–37° C for 9–12 d after thawing and grown at 33° C for 2 d immediately before assay for cell growth. Untransformed 3Y1 cells were grown at 37° C until assay. Cells were cultivated in plastic Petri dishes (Falcon) in Vogt and Dulbecco's modification of Eagle's MEM (ref. 17) with a glucose concentration of 1 g l^{-1} supplemented with, unless otherwise specified, 10% foetal bovine serum in a humidified incubator flushed with a CO₂-air mixture.

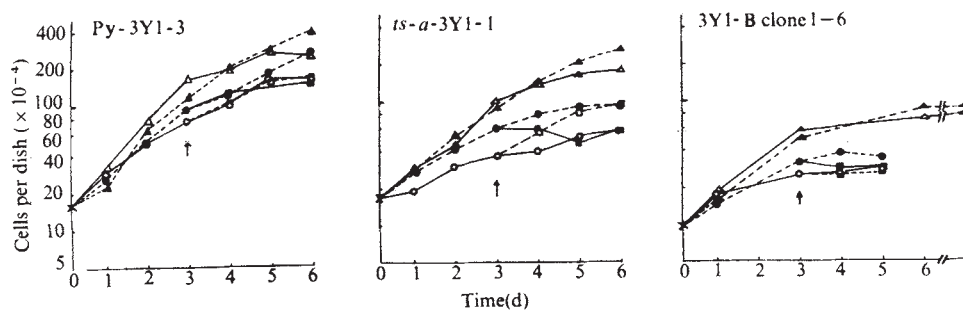


Fig. 2 Growth curves at 40° C and 33° C at different serum concentration and effect of temperature-shifts on the growth of 3Y1, polyoma-transformed 3Y1 and *ts-a*-transformed 3Y1 cell lines. Cells grown at 33° C (transformed lines) and 37° C (3Y1-B 1-6) were dispersed with trypsin-Versene, pelleted at 1,500 r.p.m. for 5 min, and suspended in Vogt-Dulbecco medium containing 1.5% foetal bovine serum and 10 units ml⁻¹ of mycostatin (Squibb). Aliquots of the cell suspension (5 ml, 2×10^5 cells) were inoculated into each 50-mm plastic Petri dish. After incubation at 33° C for 3–6 h, medium was replaced with 5 ml of the same medium containing indicated amounts of foetal bovine serum and the experimental incubation started at 40° C and 33° C (zero time). On day 3 (arrow) some cultures were shifted from 40° C to 33° C and some from 33° C to 40° C. Medium was not changed throughout the incubation. At indicated times cells were trypsinised and counted. At least 200 cells were counted for each point. ○, 2% serum at 40° C; △, 10% serum at 40° C; ●, 2% serum at 33° C; ▲, 10% serum at 33° C; □, 2% serum from 40° C to 33° C; ■, 2% serum from 33° C to 40° C.

Table 1 Virus production by rat 3Y1 cells transformed by *ts-a* mutant of polyoma virus on cell fusion with BALB/3T3 cells*

Cell line	Virus titre (plaque forming units per 10 ⁶ transformed cells)		
	Plus none (0 time)†	Plus BALB/3T3	Plus BALB/3T3 and Sendai
<i>ts-a</i> -3Y1-1	< 5	< 5	< 2.5 × 10 ¹
<i>ts-a</i> -3Y1-2	0	< 2.5 × 10 ¹	4.7 × 10 ³
<i>ts-a</i> -3Y1-3	0	1.6 × 10 ³	2.2 × 10 ⁴
<i>ts-a</i> -3Y1-4	0	2.5 × 10 ¹	1.0 × 10 ⁴
<i>ts-a</i> -3Y1-5	0	2.0 × 10 ³	< 2.5 × 10 ¹
<i>ts-a</i> -3Y1-6	0	< 2.5 × 10 ¹	1.5 × 10 ⁴
<i>ts-a</i> -3Y1-7	ND	ND	ND

*Transformed cells (10⁶) to be fused were mixed with 10⁶ BALB/3T3 cells in 0.5 ml Vogt-Dulbecco medium. The mixture was added to 0.5 ml of the allantoic fluid containing 4,000 haemagglutinating units of ultraviolet-inactivated Sendai virus (HVJ-Z strain, Y. Okada). After occasional gentle shakings at 4–10° C for 10 min, and subsequent incubation at 37° C for 30 min without shaking, the mixture was poured into a 50-mm Petri dish with 4 ml of medium. Incubation was carried out at 33° C for 6 d with a medium change on day 1. Cells were broken open into the medium by freeze-thawing and virus was titred by plaque formation on BALB/3T3 monolayer at 33° C and 39° C.

†Transformed cells (10⁶) suspended in 2.5 ml Eagle's MEM were broken open by freeze-thawing more than five times and were assayed for virus by plaque formation at 33° C using ten Petri dishes for each transformed line.

ND, not determined.

3Y1-1) as well as those of a WT-transformed 3Y1 line (Py-3Y1-3) and of untransformed 3Y1. Growth of *ts-a*-3Y1-1 cells was again very slow in 2% serum at 40° C. When cells grown in 2% serum were shifted from 40° C to 33° C and from 33° C to 40° C, resumption and slowing down, respectively, of the growth of *ts-a*-3Y1-1 cells occurred within a 24 h period, suggesting that the temperature-sensitive reaction is reversible.

As we used a cloned population of 3Y1 cells, from which all the transformed lines studied were derived, it is unlikely that WT and *ts-a* viruses are transforming different variants in the same cell population. It has already been shown that ten out of eleven 3Y1 cell lines independently transformed by WT polyoma do not produce virus spontaneously, at least during their early passages, and are free of infectious polyoma (G.K., A. Itagaki and J. Summers, unpublished). Eight of the ten virus-free transformed lines including the three lines used in this study produce virus on fusion with permissive mouse cells (G.K., A. Itagaki and J. Summers, unpublished). The *ts-a*-3Y1 lines were tested for their ability to produce virus on fusion with BALB/3T3 cells. Table 1 shows that infectious virus was not detected in extracts of all six of *ts-a*-3Y1 lines using the direct plaque assay. When cocultivated with BALB/3T3 cells, three *ts-a*-3Y1 lines produced a small amount of virus. When the cocultivation was carried out in the presence of Sendai virus inactivated by ultraviolet light, four of the six *ts-a*-3Y1 lines tested produced a relatively large amount of virus, suggesting that heterokaryon formation is necessary for virus production. The virus rescued from each transformed line was identified as the originally transforming *ts-a*, as shown by its temperature-sensitive character for plaque formation and by neutralisation with polyoma virus antiserum. These results, together with the morphological characteristics of transformed colonies described above, suggest that the transformed lines studied here were actually transformed by the virus strains under study.

I suggest that the *ts-a* gene of polyoma virus controls at least one aspect of the maintenance of transformed state in certain transformed cells, that is, the ability of transformed cells to grow in low serum medium. Whether or not the *ts-a* gene determines other properties associated with the transformed state remains to be seen.

Why three of the seven *ts-a*-3Y1 lines showed the WT phenotype is not clear. It is unlikely that these lines were transformed by the revertant virus, since the virus rescued from one of the lines (*ts-a*-3Y1-4) was found to be still tem-

perature-sensitive. A possible explanation is that secondary cellular alterations occurred during the passages of cells after the initial transformation event. Second, the virus may have transformed, by chance, pre-existing variants which might even have arisen by way of the small number of passages of the parental clone of 3Y1 cells. Third, the *ts-a*-3Y1 cells which are not temperature-sensitive might contain more viral gene copies than do the temperature-sensitive cells, so that they would have more gene product. Where there is a lot of product the cell line might function partially at 40° C, so that these cells would not be temperature-sensitive. It might also be possible that the *ts-a* gene product could interact with more than one factor or receptor site in transformed cells. The nature of the mutational change in the gene product and the nature of the cellular factor would both determine the success of the interaction.

The *ts-a* function is known to be required for initiation of each new round of polyoma viral DNA replication in productive infection^{19,20}. The same gene function seems to control both the initiation of transformation¹¹ and at least one aspect of maintenance of the transformed state. Transformation could be a consequence of the introduction into a cell of the capacity for aberrant initiation of DNA replication. A similar situation has been found and discussed more extensively as a result of studies of the SV40 mutants which have properties similar to those of the polyoma *ts-a* (refs 6, 7, 10 and 21).

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Density and dose-response curve of acetylcholine receptors in frog neuromuscular junction

THE description of the reaction between acetylcholine (ACh) and receptors in the end-plate membrane requires determination of the dose-response curve. The most appropriate response to measure is the conductance change of the voltage clamped end-plate^{1,2}. Recent improvements of the microiontophoretic method^{3,4}, as well as direct visual control of the ACh-pipette in relation to the nerve terminal⁵, enabled us to use the focal application method in a reasonably quantitative way. Only

end-plates with a sole, straight running terminal were chosen for the experiments³⁻⁵, and ACh was always applied at the end of a terminal. The known geometrical arrangement allowed us to calculate the local ACh concentration in amplitude and time course at each point of the terminal by the diffusion law, and to compare it with the measured response of the voltage clamped end-plate. Our results indicate that at least three molecules of ACh react with one receptor unit.

If r is the distance between the site of ACh release and a location x on the terminal, then the local ACh concentration C is given as a function of time t by^{6,7}

$$C = [Q/8(\pi Dt)^{3/2}] \exp(-r^2/4Dt); \quad r^2 = x^2 + z^2 \quad (1)$$

where Q is the number of molecules released at $t = 0$, $r = 0$, D the ACh-diffusion coefficient, z the distance of the ACh-pipette to the end of the terminal. It is assumed that the interaction of ACh with the receptors, at each local site, can be described by the law of mass action (n th-order reaction)^{1,2}

$$y = 1/(1 + (K/C)^n) \quad (2)$$

where y is the fraction of receptors occupied at steady state, K a constant and n the Hill coefficient. If the local conductance change, g , is proportional to the occupancy y , $g = g_{\max}y$, then the summed response, g_{sum} , of all the distributed receptors can be described as

$$g_{\text{sum}} = \int_0^l g dx = g_{\max} \int_0^l \frac{1}{1 + (K/C)^n} dx \quad (3)$$

$g_{\max}$ is the maximal conductance per micrometre length of the terminal, l the length of the terminal. This equation takes

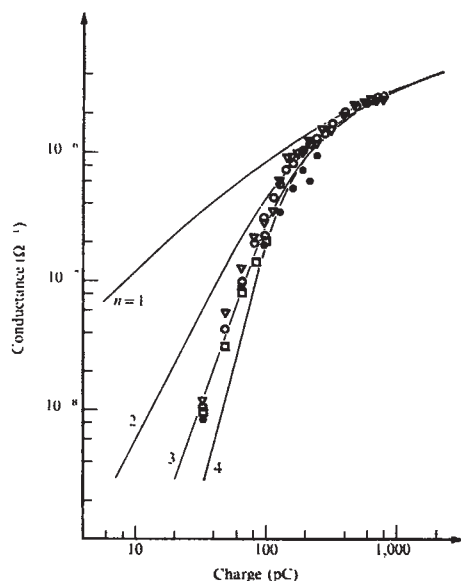


Fig. 1 ACh dose-response curve for a voltage clamped frog neuromuscular junction. Symbols, measured peak values of end-plate conductance change, for ACh released 10 μm above the end of a linear terminal. The ACh pipette was positioned four times (different symbols). Holding and resting potential -85 mV . Two intracellular micropipettes were used for a 'point' voltage clamp. The conductance change of $10^{-6}\Omega^{-1}$ corresponds to 70 nA of end-plate current. Calculated curves according to the equations given in text. Ordinate, conductance change of end-plate; abscissa, charge through iontophoretic pipette. Temperature, 23°C.

into account that with increasing doses more distant receptors contribute to the response. The integral can only be solved numerically. The calculated peak values of g_{sum} were plotted (log-log) against the dose Q . This resulted in standard dose-response curves for a linear terminal, which have a limiting slope of n for low doses, but for high doses show an increase of the response with $Q^{1/3}$ instead of saturation. The shapes of the standard curves are independent of the respective K and $g_{\max}$. Thus, experimental data can be approximated by

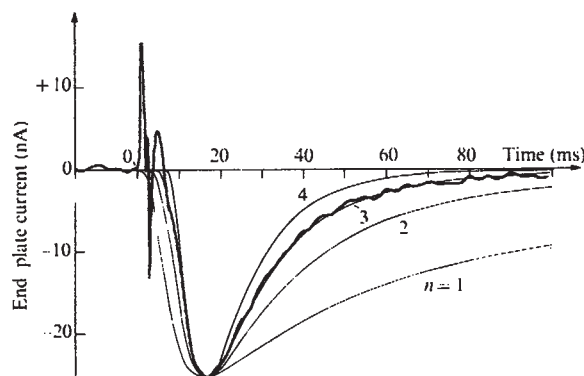


Fig. 2 Time course of measured end-plate current (—, pen recorder readout from digitally stored response). One response of those plotted in Fig. 1. Charge through ACh-pipette 120 pC (80 nA, 1.5 ms). Record starts with an artefact from the ACh-pulse. —, Theoretical responses $g_{\text{sum}}(t)$ according to the equations given in text. The curves were plotted by coinciding peak time and peak amplitude.

shifting the standard curve until the best fit is obtained, and $g_{\max}$ and K (concentration for 50% activation of $g_{\max}$) can be determined.

The result of an experiment where ACh was released 10 μm above the end of a linear terminal of a voltage clamped end-plate is illustrated in Fig. 1. The limiting slope at low doses (for determination of n) and the bend into the saturation slope (for determination of $g_{\max}$) are easily seen on the curve. The experimental data are well described by equation 3 (solid line), assuming $n = 3$, $K = 2.6 \times 10^{-5}\text{ M}$ (if ACh carries 25% of the charge driven through the pipette⁸), and $g_{\max} = 18.3 \times 10^{-8}\Omega^{-1}\mu\text{m}^{-1}$. For comparison, the standard curves are shown for $n = 1, 2$ and 4, respectively. In eight further experiments, K was almost constant (2.0×10^{-5} – $2.9 \times 10^{-5}\text{ M}$), whereas $g_{\max}$ depended on the individual end-plate (6×10^{-8} – $20 \times 10^{-8}\Omega^{-1}\mu\text{m}^{-1}$), presumably as a result of variation in the density of junctional folds (0.5–3 per μm length)⁹.

The determination of n depends very critically on the assumption that the released ACh is proportional to charge passed through the pipette. But the value of n can also be obtained by a different experiment, independent of the pipette's properties. If a single pulse is released from the pipette, the measured time course of the conductance change can be compared with the calculated $g_{\text{sum}}(t)$, according to equation (3). This is done in Fig. 2. The excellent coincidence of the theoretical curve ($n = 3$) with the measured response in both the declining and rising phase of end-plate current strongly supports the $n = 3$ conclusion of the dose-response curve. In addition, the successful description of the conductance time course justifies the earlier assumption of steady state between ACh concentration and receptor occupation in the experimental condition used. In other experiments the theoretical approximation was not as good as in the example illustrated, but in all cases the assumption of $n = 3$ clearly gave the best description.

Since the theoretical approximations of the experiments provided us with an average value of $g_{max} = 12 \times 10^{-8} \Omega^{-1} \mu m^{-1}$, we can calculate the number N of ionic channels per micrometre length of the terminal. Let γ be the conductance of a single channel, then $g_{max} = N\gamma$, since it is reasonable to assume that the closed time of the channel is short compared with the open time. The value of γ can be derived from measurements of ACh induced conductance fluctuations in the voltage clamped end-plate¹⁰⁻¹³. With $\gamma = 18.5 \times 10^{-12} \Omega^{-1}$ in the normal muscle fibres¹³, the density of ionic channels is, on the average, $6,500 \mu m^{-1}$. Since a terminal is about $2 \mu m$ wide and since receptors seem to be confined to the openings of the folds^{14,15}, and possibly occur in patches^{9,16-18} covering perhaps 50% of these regions, it is estimated that the 'active' surface is about $1 \mu m^2$ per micrometre length of terminal. Then the density of ionic channels is, on the average, $6,500 \mu m^{-2}$ at these regions of the postsynaptic membrane, a value quite close to observations of particle density in the respective patches obtained from freeze-etching studies^{9,17}. Calculations of ACh-binding sites based on measurements by autoradiography^{14,15,19} are within this range.

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sublethal dose ($150 \mu g kg^{-1}$) of 3H -diacetyl α bungarotoxin⁴ dissolved in 1 ml normal saline was injected into the thoracic cavity of Long Evans rats (body weight about 220 g) and the animals held in upright position for several hours. Both hemidiaphragms were isolated between 4 h and 5 d after toxin-injection, washed for 3 h with Tyrode solution at 37° C to remove the non-specific binding, cut parallel to the end-plate zone into three segments and the radioactivity in the central segment containing end-plates and the other two segments (non-end-plate) counted as previously described⁴. No difference was found in the binding of labelled toxin between both sides of hemidiaphragms. In the diaphragms isolated 4–9 h after injection of toxin, all the acetylcholine receptors existing on the end-plate were bound with the toxin, as further treatment of the muscle *in vitro* with $1 \mu g ml^{-1}$ of the labelled toxin for 2 h at 37° C, a condition known to saturate the receptors⁴, did not increase the binding of labelled toxin. As illustrated in Fig. 1, a sub-

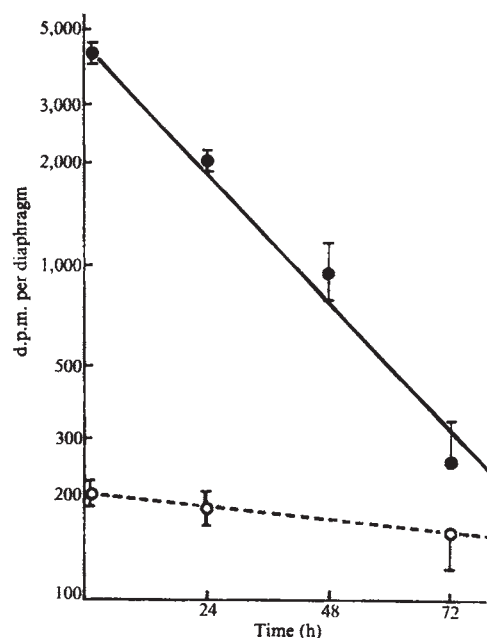


Fig. 1 The decline in 3H - α bungarotoxin bound to junctional and extrajunctional acetylcholine receptors. 3H - α bungarotoxin ($150 \mu g kg^{-1}$) was injected into the thoracic cavity of normal or denervated (9 d) rats. Radioactivity (d.p.m.) in the motor endplate zone of the diaphragm (junctional) is shown for the normal animal ($\circ$) and that in the whole muscle fibre (extrajunctional, counts on the end-plate subtracted) is shown for the denervated rats ($\bullet$) at various times after injection of the toxin. The values at zero time were obtained by saturating the receptors *in vitro* with the labelled toxin. Mean $\pm$ s.e. ($n = 6-9$).

Turnover of junctional and extrajunctional acetylcholine receptors of the rat diaphragm

THE junctional acetylcholine receptors on the motor end-plate mediate the transmission of nerve impulses to skeletal muscle, and their number per endplate is normally kept constant at $1.4-4.7 \times 10^7$ for various vertebrate muscles¹⁻⁴. In some conditions, as in rats chronically treated with neostigmine^{5,6} or in myasthenia gravis⁶, the number of acetylcholine receptors is markedly decreased; whereas, like denervation, insufficiency of neuromuscular transmission caused by various blocking agents, such as hemicholinium-3 and β bungarotoxin, tends to increase the number of receptors both at end-plate and non-end-plate zones (C. C. C., S. T. Chuang and M. C. H., unpublished). We, therefore, examined the turnover of acetylcholine receptors at end-plate and non-end-plate zones (extrajunctional) to shed more light on the pathophysiology of skeletal muscle.

The specific, and apparently irreversible, binding of α bungarotoxin isolated from the venom of *Bungarus multicinctus*⁷ made this study feasible. To bind selectively the receptor of the diaphragm without killing the animal, a

stantial amount of labelled toxin remained bound to receptors 1–3 d after injection of toxin. It is obvious that the end-plate radioactivity declined exponentially at a very slow rate (half-time calculated as 7.5 d). The radioactivity in the serum, 9 h after injection of toxin, was very low and treatment of normal diaphragms with this serum did not show appreciable binding on the end-plate area. It is therefore unlikely that the slow decay of end-plate radioactivity was caused by continuous binding of circulating toxin with free acetylcholine receptors. The question arises whether the decline of radioactivity indeed resulted from turnover of the junctional receptors, from metabolic decomposition of the bound toxin, or from dissociation of the toxin from the receptor. Although the action of α bungarotoxin is considered practically irreversible, the rate of dissociation may be so slow that it has not been detected in previous studies.

Actinomycin D (0.5 mg kg^{-1}) was therefore administered simultaneously with the labelled toxin. This treatment depressed the generation of extrajunctional receptors in the rat diaphragm after denervation but not the binding of toxin to the existing receptors in the motor end-plate⁸. Interestingly, more radioactivity was found 5 days after toxin plus actinomycin D injection than in the untreated control (Table 1), indicating that the decline of radioactivity was retarded. Although this small inhibition by actinomycin D may not be sufficient to rule out the possibility of slow dissociation of toxin from the receptor, it does suggest that the decline in radioactivity may result partly from turnover of the junctional receptors. In other experiments, a motoneurone blocking agent, β bungarotoxin⁹ ($50 \mu\text{g kg}^{-1}$), was injected together with the labelled α bungarotoxin or the phrenic nerve was cut immediately before administration of the labelled toxin. As shown in Table 1, the radioactivity remaining 5 days after labelling was less than in the control. Since both denervation^{1,3,4} and treatment with β bungarotoxin (C.C.C. and M.C.H., unpublished) markedly increase the synthesis of new acetylcholine receptors, the more rapid decline of radioactivity in these conditions may be related to an increased turnover.

Table 1 Effects on the decline of ^3H - α bungarotoxin bound to junctional acetylcholine receptors

	Radioactivity (d.p.m. $\pm$ s.e.)		
	Central segment (A)	Outer segment (B)	End-plate (A-B)
Control ($n = 7$)	98.5 ± 8.7	32.8 ± 4.0	64.9 ± 8.0
Actinomycin D ($n = 4$)	102.9 ± 7.7	1.9 ± 1.8	$100.0 \pm 7.9^*$
β bungarotoxin ($n = 5$)	61.4 ± 10.4	28.8 ± 7.1	$33.1 \pm 4.7^*$
Denervation ($n = 4$)	91.4 ± 2.9	51.8 ± 4.9	$40.2 \pm 4.4^*$

The labelled toxin was administered as described in Fig. 1 and the radioactivity remaining bound was measured 5 d after injection. Actinomycin D (0.5 mg kg^{-1}) or β bungarotoxin ($50 \mu\text{g kg}^{-1}$) was given together with the labelled toxin. For denervation, the left phrenic nerve in the neck was cut just before injection of toxin.

* $P < 0.05$ against control.

If one assumes that an acetylcholine receptor occupied by α bungarotoxin has the same half life as an intact receptor, one may take the half-time of disappearance of labelled toxin from the end-plate segment as the turnover of the junctional receptors. The value of 7.5 d obtained in this experiment, however, could be an underestimate since the decline in radioactivity may result partly from dissociation of the toxin as well as from metabolism. The decrease in number of junctional receptors to about half within 7 d in rats chronically treated with neostigmine and the subsequent restoration to normal in 5 d after withdrawal of the anticholinesterase³, suggest that the turnover of receptors may be varied under altered neuromuscular transmission.

The turnover of extrajunctional receptors was studied by examining the receptors newly generated on the diaphragm muscle after sectioning of its phrenic nerve. After 9 d of denervation, when the receptor number almost reached maximum⁴, the rats were injected with the labelled toxin as described above for the normal animal. The toxin-binding sites of the denervated diaphragm at the time of injection was determined by treating diaphragms *in vitro* with $1 \mu\text{g ml}^{-1}$ of the labelled toxin for 2 h at 37°C . The radioactivity which remained bound to the whole denervated diaphragm 1–3 d after labelling is shown in Fig. 1. In contrast to the junctional receptors, the decline in radioactivity in the extrajunctional regions occurred at a much more rapid rate. The half-time was only 19 h. Berg and Hall¹⁰ reported a half-time of about 8 h in cultured diaphragms denervated for 4 days and the decline was inhibited by cycloheximide added to the culture medium. The shorter half-time in their experiment could result either from the

difference in turnover *in vivo* and *in vitro* or the different time of experiment after denervation.

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Effects of 'calcium ionophore' X537A on frog skeletal muscle

CALCIUM ions are critically involved in the cholinergic neuromuscular transmission process. Presynaptically, Ca^{2+} is required in transmitter release¹ while postsynaptically, it plays a role in acetylcholine-receptor kinetics². Furthermore, Ca^{2+} sets off a chain of physicochemical events which leads to muscle contraction³.

The carboxylic antibiotic X537A (ref. 4) has been applied to a number of Ca^{2+} -dependent biological systems (see ref. 6 for review). The important feature of such investigations is that X537A can complex divalent cations and thereby facilitate their diffusion through organic solvents⁵ and black lipid membranes⁶. For these reasons, X537A has been used to study the role of Ca^{2+} in neuromuscular systems. Levy *et al.*⁷ have shown that X537A, when applied to rat diaphragm, results in an increase in resting and twitch tensions, which they correlated with X537A-induced release of Ca^{2+} from the sarcoplasmic reticulum (SR). Kita and Van der Kloot⁸ have shown that X537A causes transient increases in the amplitudes of end-plate potentials and in the frequency of miniature end-plate potentials in frog skeletal muscle. These results were explained on the basis of an X537A-facilitated Ca^{2+} influx into the presynaptic nerve terminals.

As a part of our investigation of the role of Ca^{2+} in the permeability-controlling mechanism in the postjunctional membrane of frog skeletal muscle, we have made a preliminary study of the effects of X537A on resting potentials (RPs) and directly initiated action potentials (APs) in muscle fibres. Our results indicate that contrary to expectation the principal cation transported by X537A across muscle fibre membranes is Na^+ rather than Ca^{2+} .

Using established electrophysiological recording techniques, RPs and APs were obtained from frog (*Rana pipiens*) cutaneous pectoris muscles (Table 1). Experiments 1–4 show that exposure of muscles to X537A results in a 10 mV reduction in the RP. This is a steady-state condition which can be maintained for at least 2 h. Under these conditions the amplitudes of the APs are reduced by 25–30 mV, while generally their durations (as measured across the -20 mV line) are prolonged. The critical membrane potential, E_{crit} , is not significantly altered. Substitution of choline⁺ for Na^+ in the medium bathing the X537A-treated muscles results in an immediate membrane hyperpolarisation beyond control values. No APs could be generated by direct stimulation, which provides further evidence that the membrane is impermeable to choline⁺.

When, after exposure to X537A, choline⁺-substituted Ringer solution is in turn replaced with the normal Na^+ Ringer solution, the muscle fibres return to a depolarised state in which APs of reduced amplitudes can again be elicited. Figure 1 shows a typical set of recordings made from muscles bathed

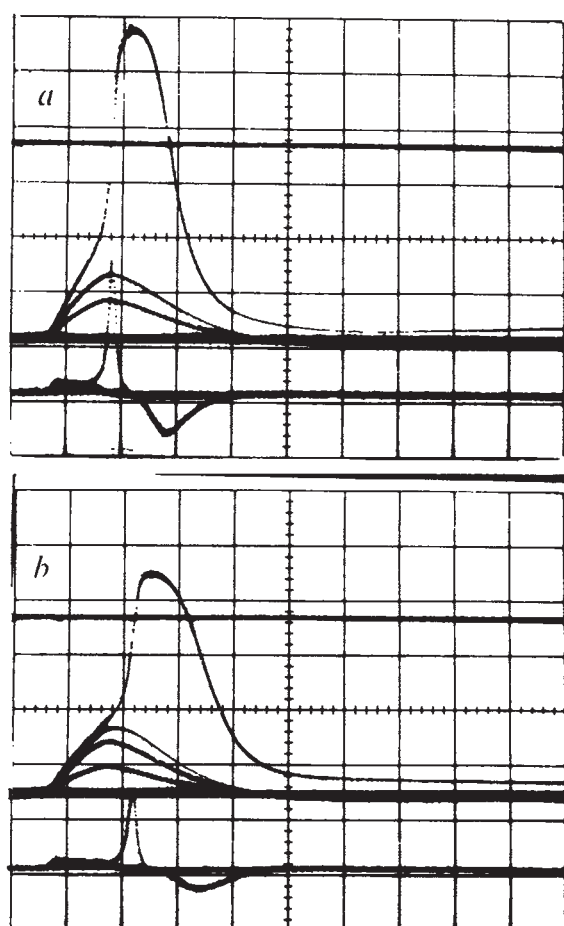


Fig. 1 Typical action potentials recorded from directly stimulated frog cutaneous pectoris muscle (stimulus duration = 1 ms). Lower traces show rate of change of membrane potential (dV/dt). Ordinate calibration, upper trace = 25 mV per division; lower trace = 250 mV per division. *a*, Control, muscle bathed in Ringer solution; first two stimuli were subliminal. *b*, Muscle bathed in Ringer solution, after 10 min exposure to 14 μ M X537A; first three stimuli were subliminal.

in HEPES-Ringer solution before and after treatment with 14 μ M X537A.

The choline⁺-substitution experiments provide strong evidence that an X537A-mediated Na⁺ influx is responsible for the observed reduction in RP. An additional experiment was carried out to test the possibility that both Na⁺ and Ca²⁺ influxes may be responsible for the depolarisation of the X537A-treated muscle fibres. In this experiment the bathing solution contained Ca²⁺ in increased concentration (composition: 108.8 mM Na⁺, 2.5 mM K⁺, 3.6 mM Ca²⁺, 117.1 mM Cl⁻, 3.0 mM HEPES buffer; pH = 7.4). Table 1 (experiment 5) shows that, with increased Ca²⁺ concentration the control values for E_{crit} became less negative. The higher Ca²⁺ concentration, however, did not significantly alter the previously observed effects of X537A on the RPs and APs. We concluded therefore that any increase in Ca²⁺ flux caused by X537A cannot be a significant factor in producing the observed membrane depolarisation.

The modest reduction in RP produced under our experimental conditions is not sufficient to account for the altered AP waveform which occurs in X537A-treated fibres. The changes in the AP may be explained by a specific interaction between X537A and the voltage-dependent Na⁺ and K⁺ channels in the muscle fibres. Tetrodotoxin (TTX) interacts with the voltage-dependent Na⁺ channels, inhibiting the conductance change which underlies the generation of the muscle AP but does not alter the muscle RP (ref. 9). Therefore, we have carried out a TTX-X537A 'competition' experiment to determine, at least in resting fibres, whether or not X537A interacts with the voltage-dependent ionic channels.

Resting potentials and APs were obtained for a muscle bathed in the following sequence of solutions: (1) normal HEPES-Ringer solution (controls); (2) HEPES-Ringer solution plus 0.32 μ M TTX; (3) 15 μ M X537A in the 0.32 μ M TTX-Ringer solution, applied for 10 min and then replaced with 0.32 μ M TTX-Ringer solution. The RPs recorded for this sequence ($n = 10$ fibres for each step) were: (1) -93.2 ± 0.9 mV in control solution; (2) -94.0 ± 0.9 mV in TTX-Ringer solution before X537A treatment; (3) -79.4 ± 1.3 mV in TTX-Ringer solution after exposure to X537A. APs could not be initiated by direct stimulation in the presence of TTX before, during, or after exposure to X537A. By comparing the RPs obtained in these experiments with those given in

Table 1 Resting potentials and action potential characteristics for frog cutaneous pectoris muscles exposed to X537A

Exp.†	<i>n</i> *	RP (mV)†	E_{crit} (mV)‡	OS (mV)§	MRR (mV s ⁻¹)¶	MRF (mV s ⁻¹)	Duration (ms)**
Controls in HEPES-buffered (Na ⁺) Ringer:							
1	6	-90.8 ± 0.8	-53.4 ± 0.9	51.3 ± 1.1	621.7 ± 11.4	180.8 ± 4.6	0.93 ± 0.02
2	8	-93.4 ± 0.9	-51.4 ± 0.6	47.3 ± 0.9	661.7 ± 6.5	140.0 ± 3.6	1.30 ± 0.02
3	10	-91.0 ± 1.0	-53.3 ± 0.6	52.5 ± 1.1	667.1 ± 18.2	185.0 ± 3.8	1.19 ± 0.02
4	9	-91.0 ± 0.6	-52.0 ± 0.6	50.8 ± 1.1	562.0 ± 3.7	163.0 ± 4.9	1.37 ± 0.00
5††	10	-90.4 ± 0.8	-45.2 ± 0.6	46.6 ± 0.9	603.3 ± 13.6	139.4 ± 2.7	1.20 ± 0.01
In HEPES-buffered (Na ⁺) Ringer, after 10 min in 14 μ M X537A solution:							
1	8	-80.5 ± 1.2	-52.8 ± 1.2	23.1 ± 1.4	348.8 ± 23.1	76.2 ± 11.5	1.23 ± 0.08
2	6	-82.3 ± 2.8	-49.6 ± 1.4	22.2 ± 0.6	417.5 ± 20.2	108.0 ± 5.8	1.26 ± 0.05
3	8	-81.4 ± 0.7	-53.3 ± 0.8	22.9 ± 1.0	395.7 ± 19.7	114.3 ± 5.7	1.27 ± 0.02
4‡‡	10	-77.9 ± 0.9	-48.6 ± 0.9	18.2 ± 0.9	286.2 ± 16.9	62.5 ± 1.9	1.78 ± 0.04
5‡‡	16	-79.3 ± 0.8	-42.4 ± 0.5	22.0 ± 1.0	348.2 ± 10.0	64.0 ± 1.9	1.46 ± 0.02
In choline ⁺ -substituted Ringer:							
1	7	-97.7 ± 1.1	—	—	—	—	—
2	10	-94.2 ± 0.9	—	—	—	—	—
3	14	-95.9 ± 1.0	—	—	—	—	—
4	10	-93.5 ± 1.1	—	—	—	—	—

*Number of records; †resting potential; ‡critical membrane potential; §overshoot of AP; ¶maximum rate of rise of AP; ||maximum rate of fall of AP; **duration of AP, measured at -20 mV line.

††Experiment 5 conducted in the presence of high (3.6 mM) extracellular Ca²⁺ throughout.

‡‡15 μ M X537A was used.

Muscles were bathed in HEPES-buffered Ringer solution (composition: 112.4 mM Na⁺, 2.5 mM K⁺, 1.8 mM Ca²⁺, 117.1 mM Cl⁻, 3.0 mM HEPES buffer; pH = 7.4). The muscles were then bathed for 10 min in HEPES-buffered Ringer solution containing 14 μ M X537A (the X537A was first solubilised in ethanol). The X537A solution was then removed and the muscle washed several times with HEPES-Ringer solution to remove the ionophore from the bath. Thereafter, with the muscle bathed in HEPES-Ringer solution, RPs and APs were again determined. Finally, Na⁺-free choline⁺-Ringer solution (composition: 112.4 mM choline⁺, 2.5 mM K⁺, 1.8 mM Ca²⁺, 117.1 mM Cl⁻, 3.0 mM HEPES buffer; pH = 7.4) was substituted as the bathing medium and RPs were determined.

Table 1, it can be seen that the effect of X537A on the RP is independent of the presence of TTX. If the RPs in fibres pre-treated with TTX had remained unaffected by X537A, we could have concluded that X537A alone alters the RP by changing the resting behaviour of the voltage-dependent Na^+ channels. On the other hand, had we been able to generate APs in fibres treated first with TTX and then X537A, we could have concluded that X537A competes for the same active site which TTX inhibits. Neither of these postulated results was observed. It is therefore likely, although not certain, that X537A does not interact with the voltage-dependent Na^+ channels which TTX inhibits. Instead, X537A probably provides a separate, voltage-independent Na^+ conductance.

Our finding that X537A enhances resting Na^+ influx is supported by the work of Cornelius *et al.*¹⁰, who have published values for the binding constants of various cations to X537A in ethanol. The binding constants for Na^+ and Ca^{2+} are given as $1.5 \pm 0.7 \times 10^6$ and $2.7 \pm 1.5 \times 10^5$, respectively. Thus Na^+ is preferentially bound to X537A by roughly one order of magnitude. The ability of an ionophore to facilitate ionic diffusion generally follows its ability to form a complex with that ion⁵.

In view of our findings, it may be useful to re-examine the conclusions reached in earlier investigations using X537A. If this ionophore were depolarising the rat diaphragm muscles studied by Levy *et al.*⁷ to values below E_{crit} , the contractile apparatus could be activated and tension increased without any direct action of the X537A on the SR. Similarly, depolarisation of the presynaptic nerve terminals by means of an X537A-facilitated Na^+ influx could in part account for the rapid, transient release of acetylcholine observed by Kita and Van der Kloot⁸.

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The mechanism of calcium ionophore-induced secretion from the rat neurohypophysis

THE release of neurohypophyseal hormones, whether evoked by electrical stimulation or by a high potassium concentration, is associated with an uptake of calcium from the extracellular space¹⁻⁴. It has therefore been proposed that calcium entry plays a key role in stimulus-secretion coupling in the neurohypophysis⁵⁻⁷. Recently, calcium ionophores have been described which increase calcium flux across the cell membrane and induce secretion in a number of secretory systems⁸⁻¹². In contrast, we report that although the calcium ionophore X-537A (Lasalocid, Hoffmann-LaRoche) promotes secretion from the rat neurohypophysis, there is no corresponding increase in calcium uptake; furthermore, ionophore-induced secretion

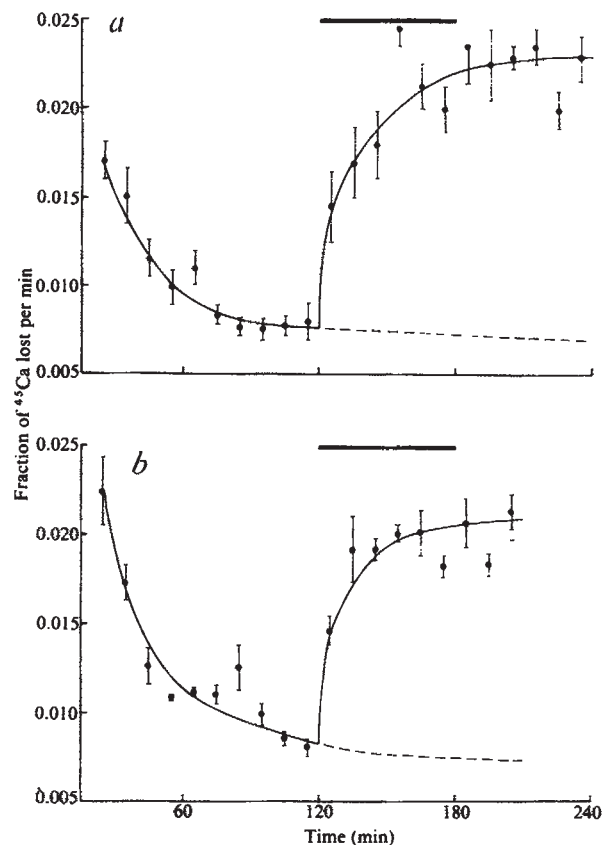


Fig. 1 Effects of X537A on the efflux of calcium from groups of four isolated neurohypophyses. The efflux was performed in a normal Locke solution (a) and in a calcium-free solution (b). X-537A ($20 \mu\text{g ml}^{-1}$) was added to the perfusate as indicated by the bars. Each point represents the mean rate constant ($\pm$ s.e.m., $n_a = 8$, $n_b = 4$) of calcium efflux observed during 240 min of washout (b, 210 min) starting at time zero. The dashed lines represent the control experiments. The bathing solution contained: NaCl, 150 mM; KCl, 5.6 mM; CaCl_2 , 2.2 mM; MgCl_2 , 1 mM; NaHCO_3 , 6 mM; glucose 10 mM. Dimethylsulphoxide (1%) was present throughout all efflux experiments. Neurohypophyses were isolated from albino rats (350-400 g body weight) and incubated at 37°C in 1 ml oxygenated Locke solution¹. After preincubation for 20 min both control and test groups of glands were transferred for 10 min to a Locke solution containing dimethylsulphoxide (1%). The glands were then exposed to the same solution containing radioactive calcium ($10 \mu\text{Ci ml}^{-1}$) for 30 min. The ionophores, diluted in dimethylsulphoxide, were present at a concentration of $20 \mu\text{g ml}^{-1}$ during the last 25 min of exposure to the label. To remove the extracellular radioactive calcium both test and control groups of glands were washed for 60 min in a normal Locke solution, changed every 10 min. The glands were then blotted, weighed, digested overnight in solune and the ⁴⁵Ca content counted. The oxytocin released into the incubation medium was determined by bioassay¹³ confirming the results already obtained¹². To study the efflux of ⁴⁵Ca from the neurohypophysis, groups of four glands were loaded for 60 min in a solution containing radioactive calcium ($33 \mu\text{Ci ml}^{-1}$) and then transferred to a solution containing non-radioactive calcium. After 10 min the solution was removed, replaced by a fresh solution and counted. This sequence was repeated for up to 240 min. Dimethylsulphoxide (1%) was present throughout all the efflux studies. At the end of the experiment the glands were digested with solune and the ⁴⁵Ca counted. When sodium chloride was removed from the solution, choline chloride was added to maintain isotonicity.

persists in the absence of external calcium ions. The most logical explanation for these results is that, rather than increasing the influx of extra-cellular calcium, the ionophore actually releases calcium from intracellular binding sites, probably mitochondria; and that it is the mobilisation of this internal calcium which, *inter alia*, promotes secretion.

Of the two ionophores tested, A23187 (Lilly) had no effect

Table 1 Effects of calcium ionophores in the neurohypophysis on calcium uptake

Experiment	^{45}Ca uptake (c.p.m. mg^{-1})
Control	443 ± 48 (8)
X537A	197 ± 21 (8)
Control	442 ± 54 (4)
A23187	410 ± 40 (4)

on either secretion¹² or calcium uptake, whereas X537A increased secretion¹² but reduced calcium uptake (Table 1). One possible explanation for this rather surprising observation is that X537A enters the cells and releases calcium from intracellular binding sites. Evidence favouring this interpretation is shown in Fig. 1. Application of X537A increases markedly the efflux of ^{45}Ca from the neurohypophysis. This may arise because X537A increases some form of internal calcium exchange either for external calcium or for external sodium ions, or because X537A raises the intracellular level of ionised calcium and this in turn is reflected in a higher rate of calcium loss from the cell. This latter interpretation seems most likely because the ionophore-induced calcium efflux persists in the absence of external calcium and sodium ions and in four experiments was still observed when ionophore was added in a solution lacking sodium, calcium and magnesium ions. Although part of the decrease in ^{45}Ca influx could be explained by a persistent increase in Ca efflux after removal of ionophore, it is possible to calculate from the efflux rate constants, that at least 37% of the observed diminution in ^{45}Ca influx must be attributed directly to ionophore action.

These data are fully consistent with the idea that the important step in initiating secretion is not simply an increased rate of calcium entry but an increase in internal ionised

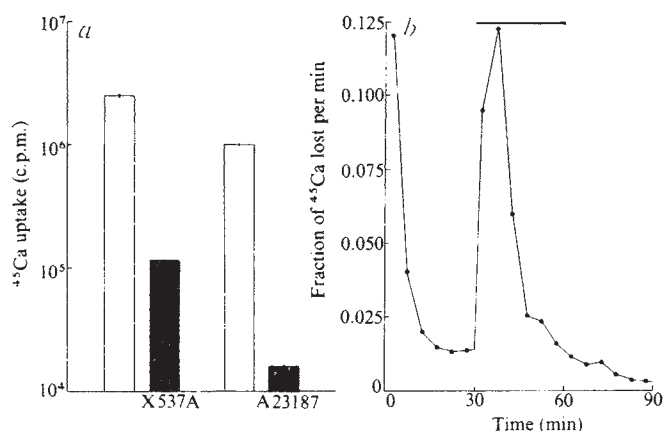


Fig. 2 Effects of calcium ionophores on calcium fluxes from isolated brain mitochondria. *a*, Uptake of ^{45}Ca into mitochondria isolated from guinea-pig brain cortex¹³. The preparation was kept in a solution containing a calcium ionophore for 3 min. ^{45}Ca was present during the last minute of exposure to the ionophore. Mitochondria were then washed on a Millipore filter and counted. White columns, control uptake (X537A control, $n = 4$; A23187 control, $n = 4$). Shaded columns, calcium uptake in the presence of X537A ($20 \mu\text{g ml}^{-1}$, $n = 4$) and A23187 ($20 \mu\text{g ml}^{-1}$, $n = 4$). Each column represents the mean $\pm$ s.e.m. *b*, Efflux of ^{45}Ca from brain mitochondria. The preparation was exposed to the label for 30 min and subsequently placed on a Millipore filter. The efflux of ^{45}Ca was determined by exposing the mitochondria to a buffer solution for 5 min followed by collecting the solution under negative pressure. This sequence was repeated at intervals of 5 min for 90 min. The amount of ^{45}Ca was measured in each sample. In addition the mitochondrial ^{45}Ca content was determined at the end of each experiment. Heavy bar, time of exposure to X537A ($20 \mu\text{g ml}^{-1}$). Buffer solution contained: KCl, 100 mM; MgCl_2 , 10 mM; Tris maleate, 20 mM; succinate, 5 mM; KH_2PO_4 , 4 mM; ATP, 2 mM; CaCl_2 , 100 μM . It was equilibrated with 5% CO_2 in O_2 .

calcium concentration, and that X537A promotes just such a rise by releasing calcium from some internal binding site. As much of the intracellular calcium in nerve cells is membrane-bound within mitochondria, these organelles are an obvious candidate as the site of action for X537A. Figure 2 shows experiments designed to test whether calcium ionophores have any effect on calcium binding by mitochondria isolated from brain. In the presence of X537A and A23187 calcium uptake is reduced and calcium efflux is increased. If these effects also occur in intact cells, they would clearly lead to an increase in ionised calcium at the expense of calcium sequestered in the mitochondria.

These results, therefore, confirm the idea that an essential step in the stimulation-secretion coupling mechanism is an increase in the internal ionised calcium concentration. Hence the effect of X537A on neurohypophyseal secretion seems analogous to that in which intracellular microinjection of calcium into nerve terminals induces an increase in the quantal release of neurotransmitters¹⁴. Finally, our results highlight the need for care in interpreting changes produced by calcium ionophores as resulting solely from a modification of the permeability of the surface membrane to calcium.

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Functional inactivation of bacteriophage λ morphogenetic gene mRNA

THE synthesis of the morphogenetic proteins of bacteriophage λ seems to be regulated at the post-transcriptional level. This conclusion is based on the observation that the ratio of protein to DNA along the left arm of the λ genome varies from gene to gene by as much as 870-fold (Fig. 1), while the ratio of mRNA to DNA in this region varies less than twofold¹, reflecting its transcription from a single promoter as a polycistronic mRNA^{2–5}. These large variations in the molar ratios of the morphogenetic proteins could be explained by three different control mechanisms. (1) The initiation of protein synthesis could be controlled at the level of ribosome binding either by initiation factor complexes or by mRNA secondary structure^{6,7}. (2) Some morphogenetic proteins could act to regulate their own translation or that of neighbouring genes (N. Sternberg, personal communication). (3) The differential translation of the late gene transcripts could be achieved by selectively inactivating some transcripts but not others. Our previous experiments using RNA:DNA hybridisation ruled out differential chemical decay of the mRNA derived from the late region of the genome, but did not exclude the possibility that differential functional inactivation of morphogenetic gene transcripts could account for

the late protein to DNA variation¹. Recent experiments indicate that functional and chemical decay are different processes which can vary widely in rate⁸ and temperature dependence⁹. Functional decay in a polycistronic message generally seems to involve an endonucleolytic attack near the 5'-end of each gene transcript^{8,10} at a specific target either in or near the ribosome-binding site¹¹. Thus mRNA inactivation is not primarily a function of the length of a transcript and can vary from transcript to transcript within the same cell¹¹. Because of this variation, differential functional decay has been invoked as a possibly significant mechanism of post-transcriptional control^{11,13}.

In this report we examine the possibility that differential functional mRNA inactivation accounts for the post-transcriptional regulation of λ morphogenetic protein synthesis. To do this we have measured the functional half lives of individual late gene transcripts. We conclude that differential functional mRNA decay is not a significant factor controlling the translation of the morphogenetic genes of λ .

The rate of chemical decay of late λ mRNA was determined in a pulse-chase experiment by measuring the loss of labelled morphogenetic gene transcripts which hybridised to λ hⁱ80 DNA as described in the legend to Fig. 2a. The results shown in Fig. 2a indicate that λ morphogenetic gene transcripts decay exponentially with an apparent half life of 2.5 min, after a 4-min lag.

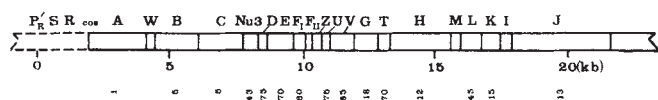


Fig. 1 Physical and genetic map of the morphogenetic region of λ showing the relative number of copies of the different late proteins. The length of each gene and its position on the map is taken from Murialdo and Siminovitch²³ and Hendrix and Casjens²⁴. The morphogenetic genes are transcribed from left to right from the late promoter P_R^{3} . Map distances measured from the late promoter are given in kilobase pairs (kb). The numbers below each gene represent the relative number of copies of protein synthesised per cell, normalised to a value of 1 for the A protein. The absolute value for the A protein is estimated to be 320 molecules per cell²³.

This measured half life of 2.5 min is only slightly longer than the chemical half life for bulk *E. coli* mRNA^{12,16}, indicating that late λ mRNA is not unusually stable, as is the case of several other phage mRNAs including T7¹⁷, T4¹⁸, S13¹², M13¹⁹ and Φ X174²⁰. The reason for the 4-min delay in the onset of degradation is unclear. Because of the presence of an excess of unlabelled uridine and rifampicin during the chase, it seems unlikely that the lag represents a balance between the decay of pre-existing labelled RNA and the continued synthesis of newly labelled RNA. If, however, mRNA degradation involves primary endonucleolytic attack followed by secondary exonucleolytic hydrolysis, then one would expect a lag between the onset of degradation and the time at which the mRNA fragments become too small to form stable hybrids with λ hⁱ80 DNA (ref. 21).

This latter interpretation is consistent with existing evidence that late λ mRNA undergoes extensive endonucleolytic cleavage (D. Schlessinger, personal communication)^{2,4,5}.

The functional half life of late λ mRNA *in vivo* was measured by determining its ability to direct the synthesis of morphogenetic proteins after inhibiting transcription with rifampicin. The decrease in the rate of incorporation of ³⁵S-methionine at various times after addition of rifampicin 30 min after infection is shown in Fig. 2b. In a control experiment in which rifampicin was omitted, the amount of ³⁵S-methionine incorporated in a 1-min pulse was relatively constant for at least 15 min, indicating that continued transcription was balanced by inactivation of mRNA. The addition of rifampicin perturbed this steady state,

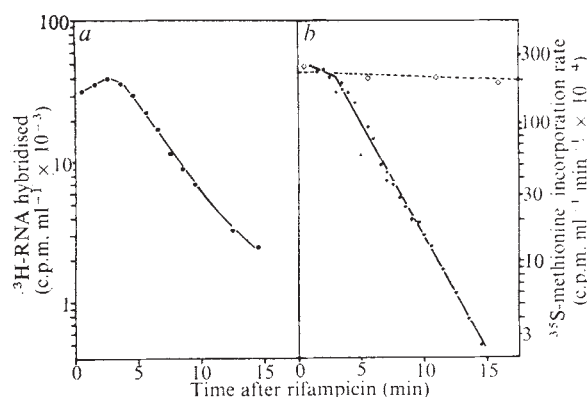


Fig. 2 Chemical and functional decay of late λ mRNA. *a*, Chemical decay. A culture of *E. coli* K12 strain 159 *her⁻thy⁻sup⁻gal⁻* was grown to 5×10^8 cells ml^{-1} in RM medium¹³. The cells were sedimented and resuspended in fresh RM medium at 2×10^8 cells ml^{-1} , ultraviolet-irradiated with $4,000 \text{ erg mm}^{-2}$ (254 nm), and infected with λ cl₁₈₅₇S_{am7} (multiplicity of infection of 5) at 0°C for 30 min¹³. The culture was diluted fivefold into warm (37°C) RM medium and incubated with aeration at 37°C. After 28 min nalidixic acid (30 $\mu\text{g ml}^{-1}$) was added to depress DNA synthesis¹⁴. ³H-uridine (New England Nuclear Corp., 30 Ci mmol^{-1}) was added to a final concentration of 50 $\mu\text{Ci ml}^{-1}$ at 30 min, and at 30.5 min labelling was stopped by the addition of rifampicin¹⁵ (Calbiochem) and unlabelled uridine to 500 $\mu\text{g ml}^{-1}$ each. Samples (1 ml) of the culture were removed at 1-min intervals after addition of rifampicin and uridine, and the cells were lysed by adding sodium dodecylsulphate to 1% w/v and heating to 100°C for 2 min²⁵. The lysates were cooled to room temperature and deproteinised by two successive phenol extractions. The RNA was precipitated with cold ethanol, dissolved in $2 \times \text{SSC}$ and hybridised to an excess of λ hⁱ80 DNA bound to nitrocellulose filters²⁶. Only RNA from the morphogenetic region of λ hybridises to this phage DNA²⁷. Approximately 90% of the c.p.m. incorporated was specific to the late region of λ . $t_{1/2} = 2.5$ min. *b*, Functional decay. A culture of strain 159 was ultraviolet-irradiated, infected, grown at 37°C as above and split into two portions. One portion was treated with rifampicin (500 $\mu\text{g ml}^{-1}$) 30 min after infection and the other was not. The functional activity of the mRNA was determined by measuring the ability of the cells to synthesise proteins at various times after addition of rifampicin. Samples (1 ml) were withdrawn at various times and pulse-labelled for 1 min with ³⁵S-methionine (35 $\mu\text{Ci ml}^{-1}$, New England Nuclear Corp., 200 Ci mmol^{-1}). Labelling was stopped by addition of unlabelled methionine (1 mg ml^{-1}) and sodium azide (50 mM). The cells were centrifuged and lysed by resuspending in electrophoresis sample buffer containing sodium dodecylsulphate and 2-mercaptoethanol and boiling for 1 min¹³. A control experiment (data not shown) indicated that 75% of the methionine incorporated was caused by λ infection. Diamonds, trichloroacetic acid (TCA) precipitable ³⁵S-methionine incorporated by the control culture in the absence of rifampicin. ●, TCA-precipitable ³⁵S-methionine incorporated by the culture after the addition of rifampicin. $t_{1/2} = 1.7$ min.

however, resulting in a biphasic decline in the rate of ³⁵S-methionine incorporation. For the first 4 min after rifampicin addition, the rate of methionine incorporation declined slowly; thereafter the rate declined rapidly with a half life of 1.7 min. The initial slow phase of decay was presumably because of a balance between the rate of mRNA inactivation and the rate of mRNA chain elongation by RNA polymerase molecules which had initiated transcription before the addition of rifampicin. These transcripts must have been completed within 4 min after the addition of rifampicin, when the decay became exponential.

To determine the functional half lives of the individual gene transcripts, the labelled samples from the above experiment were analysed by electrophoresis on SDS-polyacrylamide slab gels (Fig. 3). The relative amounts of each of several morphogenetic proteins synthesised during the 1-min pulses were measured and plotted as a function of time after the addition of rifampicin (Fig. 4a). All the curves had the same shape with a lag period of varying lengths followed by exponential decay.

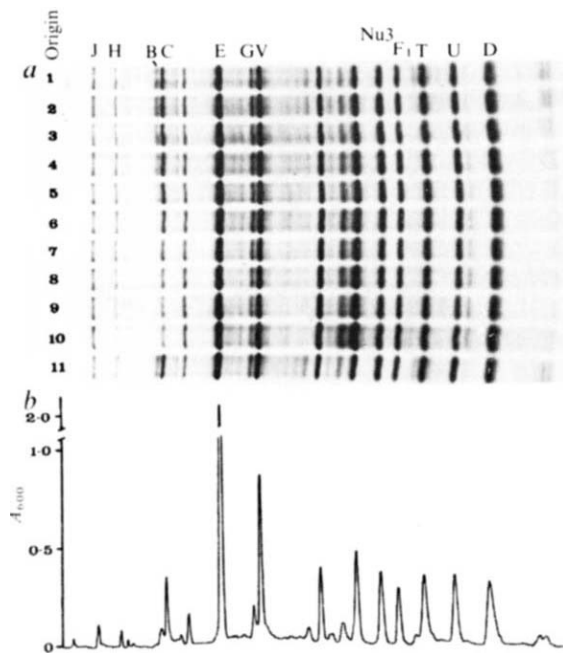


Fig. 3 Polyacrylamide gel electrophoretic pattern of λ morphogenetic proteins synthesised at different times after addition of rifampicin. *a*, Autoradiogram of a polyacrylamide gel. ^{35}S -methionine-labelled samples from Fig. 2 were analysed by SDS-polyacrylamide gel electrophoresis in slab gels containing 15% acrylamide in the separating gel²⁸. The TCA-precipitable counts loaded into each sample well were the same; the amount of material loaded increased with increasing time after addition of rifampicin as incorporation declined (compare Fig. 2). The genes coding for the various morphogenetic proteins indicated by the letters have been identified previously^{23,21}. Sample wells 1 and 11 contained extracts of labelled cells from the control cultures not containing rifampicin labelled 30 min post-infection. The other wells contained extracts of cells labelled at the various times indicated after the addition of rifampicin: 2, 0.5–1.5 min; 3, 1–2 min; 4, 1.5–2.5 min; 5, 2–3 min; 6, 2.5–3.5 min; 7, 3–4 min; 8, 3.5–4.5 min; 9, 4–5 min; 10, 4.5–5.5 min. After electrophoresis the gels were dried and autoradiographed on Kodak SB 54 X-ray film. The gels on which the other samples of Fig. 2 were analysed are not shown here. *b*, Densitometric scan of sample well 1 in the autoradiogram above. The autoradiograms from Fig. 3a were scanned on a Gilford 2400 spectrophotometer equipped with a 20-cm linear transport. Care was taken to ensure that the optical density of the band was within the linear response of the film. The areas under individual peaks were measured by weighing to determine the relative amount of labelled protein in a particular band synthesised at different times after addition of rifampicin. Previous experiments have shown that approximately 95% of the radioactive protein resolved on the gel is phage-specific²⁸.

The duration of the lag should reflect the time it takes an RNA polymerase molecule to transcribe the genes lying between the late promoter and the gene in question. If the rate of transcription is constant along the genome, then this lag is also a measure of the physical distance between the gene and the late promoter. When the lag time is plotted as a function of the distance from P_R' , we find that transcription ceases sooner than expected, especially for the distal genes *V* through *J* (Fig. 4b). The reason for the premature shut-off of transcription is not known. One possibility is that the rate of mRNA elongation is not uniform over this region of the genome. Another is that rifampicin might cause premature termination of transcription, thereby reducing the apparent delay of degradation for genes in this operon. As this effect is most obvious in the distal genes it may not have been apparent in previous studies with rifampicin which dealt with much shorter operons^{15,22}.

The functional half lives of the different morphogenetic transcripts determined from the exponential portion of the

decay curves range from 1.1 min for *C* and *J* mRNAs to 1.9 min for *D* mRNA. Since functional inactivation of each transcript occurs at an exponential rate for at least 5–6 min, it seems most likely that this inactivation is the result of random endonucleolytic attack²². Because this decay shows no apparent correlation between mRNA half life and mRNA size, the decay process probably occurs at specific sites in the transcript, even though it is random in time. For example, genes *D* and *J* differ in size by a factor of 10 while their mRNA half lives vary by a factor of 2. Although it is possible that a single inactivation event at the 5'-end of the mRNA molecule could affect all genes simultaneously, the variation in the lag before exponential decay, which varies from gene to gene, suggests that this explanation is incorrect. We prefer a model in which individual transcripts are inactivated by independent events, such as endonucleolytic cleavages at the ribosome-loading sites.

The results presented here taken together with our previous results¹ indicate that there is no preferential chemical or func-

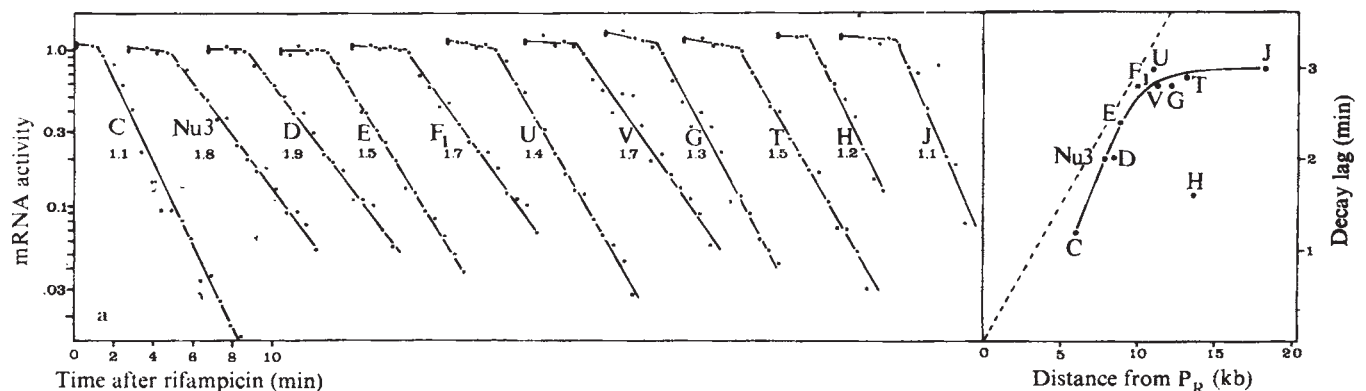


Fig. 4 Functional instability of the individual morphogenetic gene transcripts. *a*, Kinetics of inactivation after addition of rifampicin. Results are expressed as the fraction of protein synthesised in a 1-min pulse by 1 ml of the rifampicin-treated culture relative to the untreated culture. The autoradiograms in Fig. 3 were scanned and the relative amount of labelled protein in each band was measured at different times after rifampicin addition. The values obtained were corrected for the amount of sample loaded in each well of the gel. The curves for individual gene transcripts are presented in the same order as the map order and are displaced to the right to avoid confusion. The gene coding for each protein is indicated to the left of each curve together with the functional half life of the transcript in minutes. The half life was determined from the slope of the exponential portion of the curve. *b*, Lag time before exponential decay begins for each morphogenetic gene transcript. The duration of the lag phase of each curve in Fig. 4a was plotted as a function of the distance in kilobase pairs of the left end of the gene from the late promoter P_R' (see Fig. 1). The broken line represents the theoretical curve expected if the rate of RNA polymerase movement in the morphogenetic region of the genome is constant at 55 nucleotides per second²⁹.

tional decay of late λ mRNAs that could explain the large variation in the protein to DNA ratio seen in this region of the λ genome. Thus we believe that the synthesis of morphogenetic proteins is probably regulated at the level of ribosome loading mediated either by the mRNA primary or secondary structure, or by soluble factors which may be phage or host-coded.

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Control of gene expression in blue-green algae

THE blue-green algae are a major group of prokaryotes in many ways resembling, and perhaps phylogenetically related to, the chloroplasts of photosynthetic eukaryotes. Metabolic control mechanisms in this group are of interest because of its unique evolutionary position and because many of its members are obligate photoautotrophs and as such may show patterns of control different from those known in heterotrophic bacteria. Indeed, Carr and his collaborators¹⁻³ have proposed that blue-greens do not in general regulate metabolism at the level of gene expression, as heterotrophs commonly do. An exogenous metabolite may be assimilated, but its presence in the cell does not induce (or repress) the synthesis of enzymes responsible for its own catabolism (or biosynthesis). Thus, these workers argue, exogenous substrates do not usually support significant dark growth, or even stimulate growth in the light, because blue-greens simply cannot adjust the levels of the enzymes of intermediary metabolism to accommodate any source of carbon other than CO₂ or any source of energy other than light¹.

To establish whether any controls at the level of gene expression have evolved in blue-green algae, it seems pertinent to look for responses to environmental stimuli which do affect the growth of these organisms. Such stimuli include CO₂, sources of phosphorus and nitrogen, and, of course, light. Apparent induction or repression of enzyme systems responsible for assimilation of phosphate⁴, nitrate⁵ and

molecular nitrogen⁶ has been described. Here we show that removal of light provokes both quantitative and qualitative changes in the protein complement produced by the unicellular, obligately photoautotrophic blue-green alga *Anacystis nidulans*, and briefly discuss evidence for the 'dark induction' of two enzymes specifically required for dark endogenous energy metabolism.

Figure 1 shows the effect of removal of light on the rate

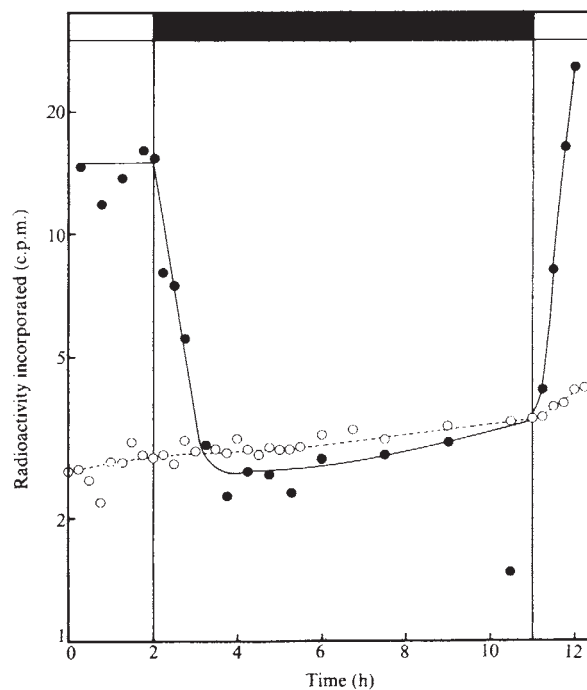


Fig. 1 Relative rates of protein synthesis in light and dark. A culture of our α -isopropylmalate synthetase deficient auxotroph, *leu201* (to be described elsewhere) was grown⁷ for 48 h (4-6 generations) in medium containing L-leucine at 10 μ g ml⁻¹ and ¹⁴C-DL-leucine (New England Nuclear) at 0.01 μ Ci ml⁻¹. Illumination was terminated at 2 h and resumed at 11 h. Samples of 1 ml were withdrawn at intervals to tubes containing 100 μ Ci ³H-L-leucine (New England Nuclear) and incubated for 15 min under the same illumination conditions as the culture. Labelling was terminated by addition of trichloroacetic acid to 5%, and radioactivity incorporated into protein determined as c.p.m. remaining acid precipitable after 20 min at 90 °C. ●, ³H-leucine incorporated during 15 min pulses; ○, ¹⁴C-leucine incorporated during continuous labelling from 48 h.

of incorporation of radioactive leucine into protein in a leucine-requiring mutant of *A. nidulans*. As there is no endogenous leucine synthesis in this strain (unpublished) and little protein turnover (unpublished), label incorporation is a direct measure of protein synthesis. Rates fell (Fig. 1) during the first hour of darkness and remained low until the culture was reilluminated. At least a dozen proteins were synthesised, however, in the dark which in the light were either not made, or made in lower relative amounts. This is apparent from Fig. 2, which presents SDS-polyacrylamide gel profiles of soluble proteins from cultures labelled for two generations in the light with ¹⁴C-DL-leucine and then (after removal of ¹⁴C) with ³H-L-leucine either for 1 h in the light (a), during the first hour after darkening (b), between the fourth and tenth hour after darkening (c), or during the first hour after reillumination (d). Several polypeptides were prominent in dark-³H-labelled material (b and c) which were not prominent in either ¹⁴C-'prelabelled' or ³H-labelled material produced in the light (a and d). Most obvious were species of apparent molecular weights 90,000,

60,000, 48,000 and 41,000 (all values $\pm 10\%$). Eight additional preferentially synthesised polypeptide chains could be identified when the ratios of ^3H to ^{14}C in each gel slice were plotted, as in Fig. 3. Two of these (130,000 and 29,000) were apparent only in material labelled during the first hour of darkness. Ratios of ^3H to ^{14}C were relatively constant when ^3H -leucine incorporation occurred in the light (a and d), although there was some evidence for preferential synthesis of material of very high molecular weight immediately after reillumination of darkened cells (d). All these patterns are reproducible, with some variation in peak height from experiment to experiment. Addition of the photosystem II inhibitor 3-(p-chlorophenyl)-1,1-dimethylurea (CMU) produced an alteration in the pattern of protein synthesis grossly similar to that provoked by darkness.

These data do not allow us to assign functions to any of the polypeptide chains produced preferentially in the dark. It is reasonable to assume that at least some of these are enzymes (or subunits of enzymes) specifically required in the dark. It is known that certain enzymes of the oxidative pentose phosphate pathway are essential only for dark (endogenous) metabolism^{8,11}. We therefore measured specific activities of three of these (glycogen phosphorylase, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase) in cultures before and after removal of light.

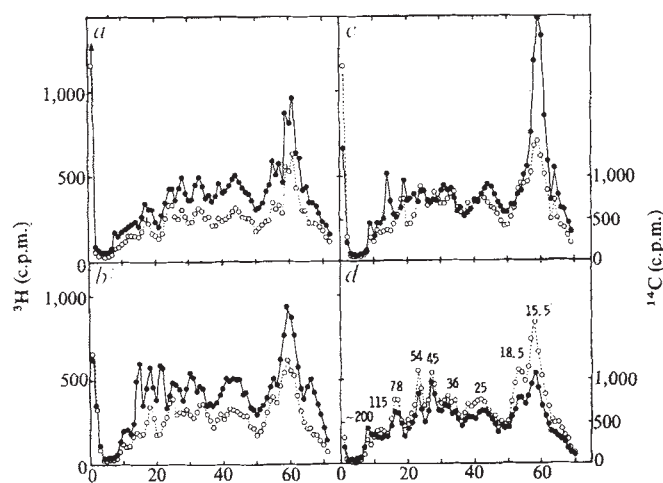


Fig. 2 Sodium dodecylsulphate (SDS)-polyacrylamide gel profiles of soluble proteins from cells labelled in light and dark. A culture of *leu201* was grown for 15 h in medium containing $10 \mu\text{g L-leucine ml}^{-1}$ and $^{14}\text{C-DL-leucine}$ at $0.1 \mu\text{Ci ml}^{-1}$. The culture was collected, washed, and resuspended in non-radioactive, leucine-containing medium. After 90 min growth to allow recovery, the culture was divided into four subcultures. Subculture a was exposed to $^3\text{H-L-leucine}$ at $0.8 \mu\text{Ci ml}^{-1}$ for 1 h in the light, while subcultures b, c and d were darkened. b was labelled ($3.8 \mu\text{Ci } ^3\text{H-L-leucine ml}^{-1}$) during the first hour after darkening; c was labelled ($3.8 \mu\text{Ci } ^3\text{H-L-leucine ml}^{-1}$) between the fourth and tenth hour after darkening; d was reilluminated after 9 h and labelled ($0.8 \mu\text{Ci } ^3\text{H-L-leucine ml}^{-1}$) during the first hour after reillumination. Each subculture was collected by centrifugation immediately after labelling, resuspended in lysis buffer^{8,11} and passed through an Aminco French pressure cell at 16,000 pounds inch⁻². Lysates were centrifuged at 17,000g for 30 min and protein was precipitated from the supernatant fluids with trichloroacetic acid at 5%. Precipitates were collected by centrifugation, washed twice with ethanol, dried and solubilised in sample buffer⁹ by heating at 100°C for 10 min. Samples were loaded on and electrophoresed through 7.5% polyacrylamide gels containing SDS⁹. Gels were sliced, solubilised in Protosol, and counted in toluene-Omnifluor (New England Nuclear) for determination of ^{14}C and ^3H radioactivity. Numbers in d indicate molecular weights (in thousands) determined with reference to a set of standard marker proteins run on parallel gels¹⁰. Qualitatively similar profiles were obtained when total lysate proteins, rather than 17,000g supernatant proteins, were treated in this way. ●, ^3H -radioactivity; ○, ^{14}C -radioactivity.

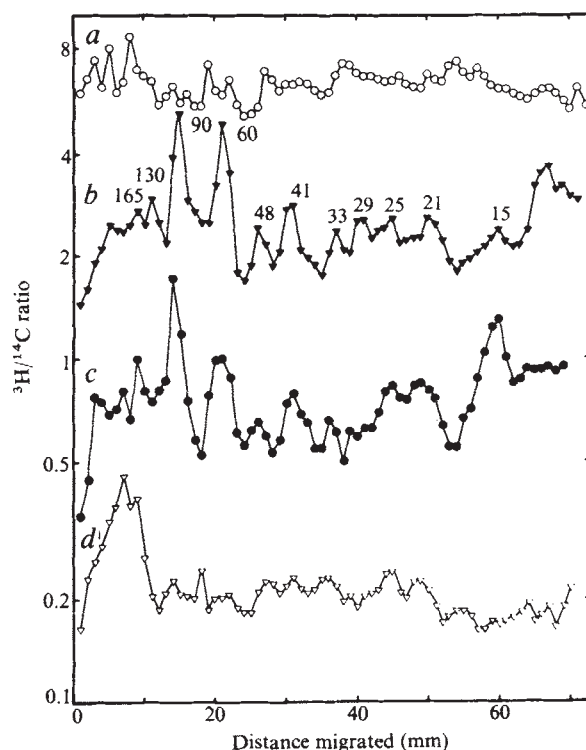


Fig. 3 $^3\text{H}/^{14}\text{C}$ ratios. The ratio of ^3H to ^{14}C radioactivity was computed for each slice of the gels represented in Fig. 2. Numbers above curve b indicate molecular weights in thousands, as described for Fig. 2. Ratios are plotted on a logarithmic scale to allow direct comparison of relative peak heights between gels.

Although levels of the third enzyme remained constant, the first two showed reproducible, chloramphenicol-inhibitable, 1.5 and 2.0-fold increases (respectively) in specific activity. These increases occurred over about one-third of a generation and at a period when overall rates of protein synthesis were rapidly falling (Fig. 1). We calculate that they correspond to increases in the differential rates of synthesis of these two proteins of at least 20-fold (unpublished).

Although blue-green algae may indeed not respond to most exogenous substrates by altering levels of enzymes responsible for their utilisation or formation, we do not think it safe to maintain, as Carr¹ has done, that blue-greens are largely incapable of exerting control at the level of gene expression. To demonstrate such control, however, it is necessary to look at environmental factors other than exogenous carbon compounds, and in particular at factors on which the growth of these organisms is known to depend.

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Location of non-DNA components of closed circular colicin E1 plasmid DNA

COLICIN E1 plasmid (Col E1) DNA usually exists as closed-circular duplex molecules of molecular weight 4.2×10^6 (ref. 1). In addition to the form that is assumed to contain only deoxyribonucleotides linked by phosphodiester bonds, two other types of supercoiled closed-circular molecules have been described. One is a DNA-protein complex, called a relaxation complex². When the protein is denatured or destroyed, the complex is converted to an open-circular structure that has a break (a nick or a gap) in the heavy (H) strand (as defined by CsCl-poly(U,G) density gradient centrifugation)³. The other type is the closed-circular molecule that accumulates in bacteria when protein synthesis is inhibited and contains a sequence of ribonucleotides (RNA) generally in either the light or heavy strand^{4,5}. The functions of these non-DNA components in replication of the plasmid have been the subject of speculation^{2,5}. Recently, it was shown that Col E1 DNA replication initiates from a fixed region which is located approximately 20% of the molecular length from the single site of cleavage of endonuclease *EcoRI*⁶⁻⁸. Replication proceeds unidirectionally⁶⁻⁸ and terminates at or near the origin of replication⁹. It was thus interesting to locate the interruption in a DNA strand resulting from either the removal of the relaxation protein or the hydrolysis of the RNA with respect to the origin/terminus region. By measuring the size by gel electrophoresis of DNA fragments formed by denaturation of linear molecules produced by the treatment of open-circular molecules containing a relaxation break with endonuclease *EcoRI*, the break was located at approximately 20% of the length from the end of the linear molecule¹⁰. We developed an electron microscopic method to determine the position of a single-stranded break and found that the relaxation break is located approximately 20% of the length from the end which has the

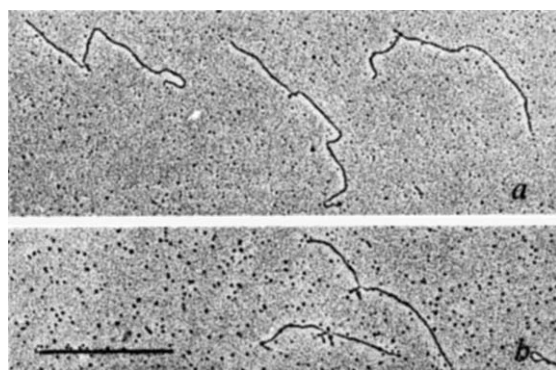


Fig. 1 Electron micrographs of relaxation complexes that had been treated successively with Pronase, exonuclease III and endonuclease *EcoRI*. Purified relaxation complexes (34S) were prepared³ from A745 (Col E1) *thy* (ref. 9) grown in a medium containing ³H-thymidine (20 mCi mmol⁻¹). After treatment with Pronase (Sigma, 200 µg ml⁻¹) for 30 min at 37° C, the DNA (10 µg in 2 ml) was layered on 36 ml of a 15 to 50% linear sucrose gradient and centrifuged in a Beckman SW 27 rotor at 27,000 r.p.m. for 24 h at 20° C. The fractions containing open-circular molecules were pooled and centrifuged in a Beckman Type 40 rotor at 36,000 r.p.m. for 20 h at 15° C. The pelleted DNA was suspended in 0.1 M Tris, pH 8.0. After addition of MgCl₂ to 2 mM the DNA was treated with exonuclease III (provided by Dr M. Fuke) to render about 10% (a) or 25% (b) of the total amount acid-soluble. The reaction was terminated by the addition of EDTA to 4 mM. After inactivation of the enzyme by heating at 80° C for 10 min and addition of 10 mM of MgCl₂, the DNA was treated with *EcoRI* (provided by Dr D. Nathans)⁶. Samples were prepared for electron microscopy⁸. The scale represents 1.0 µm.

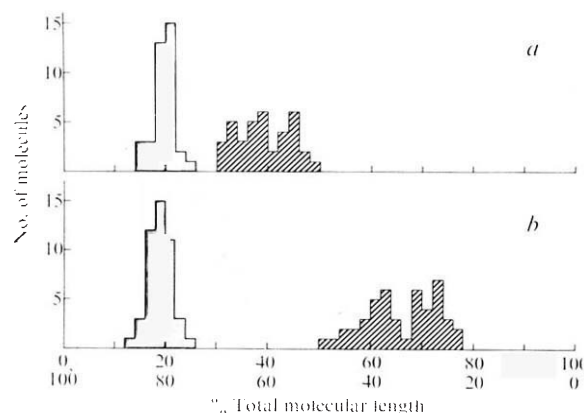


Fig. 2 Histograms showing the distribution of the fractional lengths of two double-stranded regions of molecules in the samples described in the legend to Fig. 1. The result obtained with a, sample a; b, sample b. The length of the shorter double-stranded region (filled column) is shown by the fractional length from the left in the abscissa and that of the longer double-stranded region (hatched column) from the right. The average lengths of the shorter double-stranded regions of 37 molecules in sample a and 46 molecules in sample b are 0.45 ± 0.05 µm ($20.2 \pm 2.3\%$) and 0.43 ± 0.08 µm ($19.4 \pm 3.6\%$), respectively. The average length of the longer double-stranded regions of molecules in sample a is 1.37 ± 0.13 µm ($62.0 \pm 5.9\%$) and that in sample b is 0.77 ± 0.17 µm ($34.7 \pm 7.7\%$). The total length of untreated molecules is 2.22 ± 0.09 µm (100%).

3' end of the H strand of the linear molecule created by the treatment with endonuclease *EcoRI*. On the other hand the break caused by removal of RNA is distributed at random along the molecule. Hybridisation experiments with labelled DNA fragments which were made immediately after initiation of replication to the strands of Col E1 DNA treated successively with endonuclease *EcoRI* and exonuclease III show that the relaxation break is located at the origin/terminus region.

³H-labelled relaxation complexes were converted to the open-circular forms as described in the legend to Fig. 1. After purification, they were treated with exonuclease III, which acts on the 3' end of double-stranded DNA¹¹. Two samples were prepared in which approximately 10 and 25% of the DNA was digested. After inactivation of the enzyme, the molecules were further treated with endonuclease *EcoRI* to convert them to linear structures⁶. Electron microscopic examination⁸ of these molecules showed that they contained only one single-stranded region as indicated by the formation of a bush or a thinner stretch between thicker threads in molecules spread by the ammonium acetate technique or the formamide technique, respectively. This finding indicates that the molecule contains only a single break in one of the strands. Representative molecules spread by the ammonium acetate technique are shown in Fig. 1.

To determine the location of the relaxation break, the lengths of double-stranded regions on either side of the bush in each molecule were measured. The result presented in Fig. 2 shows that the length of the shorter double-stranded region was approximately 20% of the length of the untreated molecule independent of the extent of digestion but that of the longer double-stranded region was reduced as the digestion progressed. This indicates that the relaxation break is located approximately 20% of the length from an end of the molecule created by cleavage by endonuclease *EcoRI* and the end has the 3' end of the H strand in which the relaxation break exists³. Since the origin/terminus region is located approximately 20% of the length from a molecular end formed by endonuclease *EcoRI*, the origin/terminus region and the relaxation break are closely located or they sit at symmetrical positions relative

to the *EcoRI* site. The following experiments were performed to distinguish between these two possibilities.

In a soluble system, Col E1 DNA replication proceeds through an early replicative intermediate which has a small replication loop containing a 6S DNA fragment(s)^{12,13}. Fragments of 6S DNA labelled with α -³²P-dTMP were purified and those hybridised to H strands of Col E1 DNA were isolated (legend to Table 1). The ³²P-labelled DNA was then annealed to the denatured DNAs prepared by the following procedure. First, closed-circular Col E1 DNA was cleaved by endonuclease *EcoRI* to a linear form. The linear DNA was then treated with exonuclease III to render 40% of the DNA acid-soluble. By this treatment approximately 40% of the lengths of both strands was assumed to be digested from their 3' ends because digestion by exonuclease III proceeds more or less synchronously from the 3' ends, as shown by Fig. 2. The DNA treated with endonuclease *EcoRI* and that treated with endonuclease *EcoRI* and exonuclease III were denatured and fixed on membrane filters. They were annealed to a mixture of ³²P-labelled 6S DNA (hybridised to H strands and separated from them) and ³H-reference DNA. Table 1 shows that the exonuclease-III-treated DNA had a drastically reduced capacity to hybridise the 6S DNA while it had a capacity to hybridise to the reference DNA (prepared by

shearing and denaturation of the total Col E1 DNA) corresponding approximately to the extent of digestion. The result indicates that the 6S DNA was hybridised to H strands at a region within 40% of the molecular length from the 3' end created by endonuclease *EcoRI*. Together with the result presented in Fig. 2, this proves that the relaxation break is at or near the region where replication is initiated. By its location at the origin/terminus region, the relaxation protein is probably involved directly in replication or its regulation. The absolute polarity of Col E1 DNA strands was determined by the above results as follows: the 3' end of the H strand of a linear molecule created by cleavage by endonuclease *EcoRI* is located closer to the origin/terminus region than the 5' end of the strand.



Fig. 3 Electron micrograph of molecules containing RNA which had been treated successively with RNase A, exonuclease III and endonuclease *EcoRI*. Closed-circular molecules containing RNA were prepared⁴ from A745 (Col E1) *thy* cells which had been treated with chloramphenicol (180 μ g ml⁻¹) for 15 h at 37° C in a medium containing methyl-³H-thymidine (8 mCi mmol⁻¹). After treatment with RNase A (900 μ g ml⁻¹ Worthington, heated at 100° C for 5 min) at 37° C for 30 min, open-circular molecules were isolated. Approximately 10% of the total amount of the DNA was digested with exonuclease III and the molecules were cleaved by endonuclease *EcoRI*. They were examined under the electron microscope.

Table 1 Hybridisation of 6S DNA, prehybridised to H strands, to denatured Col E1 DNA previously treated with endonuclease *EcoRI* and exonuclease III

Denatured DNA fixed on a filter	Radioactivity in hybridised DNA (c.p.m.)	
	6S ³² P-DNA	Reference ³ H-DNA (sonicated and denatured)
DNA treated with <i>EcoRI</i> (a)	163 (59%)	13,000 (62%)
DNA treated with <i>EcoRI</i> and ExoIII (40% digestion) (c)	22 (8%)	6,830 (33%)
Input DNA (c.p.m.)	275 (100%)	20,750 (100%)

³H-labelled closed-circular DNA was prepared from A745 (Col E1) *thy* cells which had been treated with chloramphenicol (180 μ g ml⁻¹) for 15 h at 37° C in a medium containing ³H-thymine (1 mCi mmol⁻¹). Purified closed-circular DNA⁴ was treated with *EcoRI* to convert more than 95% of the molecules to linear forms⁴. The reaction was terminated by the addition of EDTA to 10 mM. The linear DNA was further treated in three different ways as follows: (a) the DNA was denatured by 0.1 N NaOH followed by neutralisation by 1 N HCl; (b) the DNA was denatured by heating in the presence of poly(U,G) and centrifuged in a CsCl density gradient⁹ and the DNA in the heavy peak was dialysed and self annealed as described for the preparation of separate strands of λ phage DNA¹⁴; (c) the DNA preparation was heated at 80° C for 10 min to inactivate *EcoRI* and diluted fivefold with 0.07 M Tris-HCl, pH 8.0; after addition of MgCl₂ to 7 mM, the DNA was treated with exonuclease III to render 40% of DNA acid-soluble and then denatured by 0.1 N NaOH followed by neutralisation. The DNAs were fixed on membrane filters (Schleicher & Schuell, Bac-T-Flex B6) and treated with the preincubation mixture¹⁵. The amount of DNA fixed on a filter was approximately 2 μ g for whole DNA (a), 2 μ g for H strands (b) and 1.2 μ g for exonuclease III treated DNA (c). ³²P-labelled 6S DNA was prepared *in vitro* in a standard reaction mixture containing an extract of YS10(Col E1) cells, α -³²P-dTTP (5 Ci mmol⁻¹), glycerol and spermidine as described previously¹³. Early replicative intermediates (26S) were isolated and labelled 6S DNA was released by heating at 90° C for 2 min¹². The DNA was then annealed with a filter with fixed H strands for 18 h at 64° C in an incubation mixture (1 ml)¹⁵. The filter was washed¹⁴ and then treated with 0.5 ml of 0.05 N NaOH at 37° C for 1 h. The filter was removed and the solution was neutralised by 1 N HCl. Approximately 90% of ³²P-DNA was recovered. The prehybridised ³²P-DNA was mixed with approximately 0.1 μ g of Col E1 DNA labelled with methyl-³H-thymidine (2 Ci mmol⁻¹). The ³H-DNA was previously sonicated for 5 min in a Raytheon sonicator, denatured by 0.1 N NaOH and then neutralised by 1 N HCl. The mixed DNA solution (1 ml) was distributed in vials containing denatured DNA fixed on filters as indicated, and the mixtures were incubated at 64° C for 18 h to permit annealing. Filters were washed and radioactivity was measured. ³H-radioactivity from DNA that was first fixed to the filter was subtracted from the radioactivity obtained. The subtracted count was less than 5% of the count observed.

Closed-circular molecules which contained RNA as a result of their replication in the presence of chloramphenicol were examined next. Molecules were prepared from bacteria which had been incubated for 15 h in the presence of chloramphenicol⁴. By treating with RNase A (ref. 4) approximately 50% of the molecules were converted to open-circular molecules which were isolated by sucrose density gradient centrifugation. After digestion of approximately 10% of the open-circular DNA by exonuclease III, which is known to act also on RNA hybridised to DNA¹⁶, it was converted to linear molecules by treating with endonuclease *EcoRI*. When these molecules were examined under the electron microscope, most of them had a single bush (Fig. 3). The double-stranded regions of the linear molecules were measured and the length of the shorter double-stranded region is plotted against that of the longer one (Fig. 4). Clearly the site of the RNA is distributed at random along the molecules. This conclusion was confirmed by another method. After RNase A treatment, the DNA was phosphorylated at the exposed 5' termini with γ -³²P-ATP and polynucleotide kinase¹⁷. They were treated with endonuclease *EcoRI* and subjected to agarose gel electrophoresis after heat denaturation. The ³²P radioactivity was equally distributed in molecules of various lengths (data not shown). These results eliminate the possibility that the RNA is involved in the initiation of the replication cycle of Col E1 DNA⁴ since replication is initiated in the fixed region⁶⁻⁸ even during

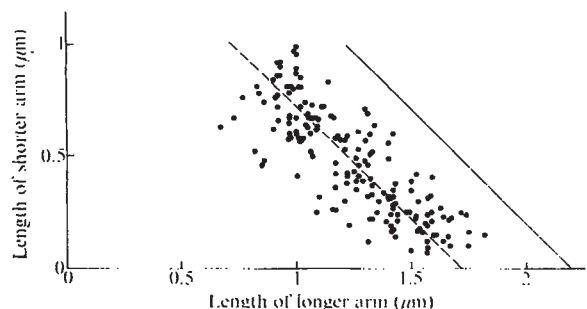


Fig. 4 The position of the bush in each molecule in electron micrographs of the sample described in the legend to Fig. 3. Each of the 175 points represents one molecule with a bush. Ten molecules with two bushes are not included. The sum of the lengths of both double-stranded regions at any point on the diagonal solid line represents the average length of untreated molecules ($2.22 \pm 0.09 \mu\text{m}$, 100%) and that on the diagonal dotted line shows the average length of the sum of double-stranded regions of a digested molecule ($1.72 \pm 0.15 \mu\text{m}$, 78%).

growth in the presence of chloramphenicol (J. Inselburg, personal communication). It is more likely that it is a remnant of RNA formed during chain elongation and is normally removed soon after its formation. Alternatively, it is still possible that the RNA is not formed during the normal process of DNA replication but resulted from the special conditions present during the chloramphenicol treatment.

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cyclic AMP²⁻⁶. We have studied the mutant *tsC42* (described previously⁷) and report that the *in vivo* expression of the *lac* operon is under the control of a phase-specific protein which is only synthesised or activated 20-30 min before cell division. This protein is essential for both the formation of membrane components and cell growth, as described previously⁷. We describe procedures for the isolation of the mutant and report its specific inability to express the *lac* operon at the transcriptional level.

The formation of cytochrome *b₁* (ref. 8), L- α -glycerophosphate uptake systems* and the β -galactoside transport systems (M.O., unpublished) occur at the same particular phase in the cell cycle of *Escherichia coli*. Furthermore, when the loss of viability of an unsaturated fatty acid auxotroph was followed after removal of the fatty acids, the cells died in a stepwise manner at a cell phase where the membrane components described above were formed (M.O., unpublished). This suggested that the corresponding processes involved in the formation of cytochrome *b₁* and so on must be concerned with other important cellular events. We therefore selected the mutant with a phase-specific defect, assuming that cell growth is temperature-sensitive and the formation of the β -galactoside transport system is under the control of the corresponding *ts*-mutation.

A derivative of *E. coli* K12; strain AT713 was treated with the mutagen nitrosoguanidine as described previously⁸ and the cells were plated on λ -agar plate⁹, supplemented with 0.1% glucose after full segregation, and incubated at 30°C. When colonies developed to a diameter of 1.5-3 mm, the plates were transferred to 42°C. After 60 min incubation, the colonies were sprayed with a solution containing 4 mM isopropyl- β -D-thiogalactoside (IPTG) and further incubated for 60 min at the same temperature. Non-inducible colonies were detected by spraying with 4 mM *o*-nitrophenyl- β -D-galactoside (ONPG) solution containing chloramphenicol (2 mg ml⁻¹). Colonies showing less or no colour development were removed and

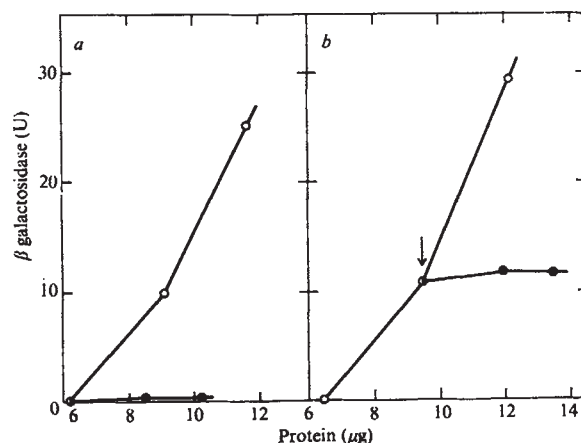


Fig. 1 Formation of β galactosidase in the mutant *tsC42*. Cells grown for several generations at 30°C in A medium¹⁰ were divided into two parts: one immediately transferred to 42°C (●), the other maintained at the same temperature (○). β galactosidase was induced by the addition of 0.2 mM IPTG at the time of culture division (a) or 30 min before culture division (b). Extreme reductions in β -galactosidase formation were observed in both cases at the non-permissive temperature. In the culture was divided at the arrow. The β -galactosidase assay was performed according to a method of Pardec *et al.*¹¹. A unit corresponds to μmol ONPG hydrolysed per h per ml. Growth is expressed as increase in protein, measured according to the method of Lowry *et al.*¹². The activities of β -galactoside transport system induced by 0.2 mM IPTG at a permissive temperature were measured at 30°C and 42°C to show the intactness of the system. These activities and the ratio of 42°C/30°C are almost identical to those of the parental strain AT713, indicating that the reduced formation of β -galactosidase at a non-permissive temperature is not a result of a decrease in the transport of the inducer (Table 1).

Regulation of expression of *lac* operon by a novel function essential for cell growth

THE study of the *in vitro* transcription of the *lac* operon using a soluble fraction of bacteria and DNA containing the genes of the *lac* operon, indicates that gene expression is controlled by both the inducer-operator interaction¹ and the action of

clones, the growth of which is temperature-sensitive, were selected using the replica method on complete agar plates. Out of 105 non-inducible clones, four strains showed the expected phenotypes; *tsC42* was one of these.

The β -galactoside transport system was very poorly formed at a restrictive temperature in the mutant *tsC42* when assayed using an *in vivo* ONPG hydrolysis according to the method of Wilson *et al.*¹⁰. It was found later that the mutant also showed no β -galactosidase activity (Fig. 1). The formation of the β -galactoside transport system was similarly arrested at a restrictive temperature (data not shown).

To find out whether the functional inability in the mutant *tsC42* occurred at the transcriptional or post-transcriptional level, we studied mRNA synthesis. DNA-RNA hybridisation experiments (Fig. 2a), performed with $\phi 80$ *plac* DNA, indicate that the amount of the *lac*-specific mRNA is considerably reduced at 42° C (about 20% of that at 30° C), in spite of the fact that the total amount of pulse-labelled RNA is almost identical at both temperatures (data not shown). The increase in β -galactosidase activity at 42° C was measured in a parallel experiment and was about 20% of that at 30° C. Such similar values for the *lac*-mRNA and β galactosidase, suggest that the functional defect in the mutant *tsC42* lies at the transcriptional level. The *trp*-mRNA synthesis, detected by hybridisation with $\phi 80$ *ptrp*-ED-DNA, is shown in Fig. 2b. The amounts of mRNA synthesised in the derepressed state at 30° C and 42° C are almost identical, in contrast to the *lac*-mRNA. Thus, *trp*-mRNA synthesis proceeds normally in a restrictive condition, transcription not usually being impaired but specific for the *lac* operon.

Cyclic AMP seems to be required for the expression of the *lac* operon¹⁴⁻¹⁷. We have therefore examined the effect of cyclic AMP (1 mM) on the formation of β galactosidase in the mutant *tsC42* and found no detectable stimulation at a restrictive temperature. Furthermore, this reagent could not restore the inability to grow at 42° C. Perlman and Pastan¹⁷ have reported that a mutant lacking adenyl cyclase is unable to grow on lactose, maltose, arabinose, manitol and glycerol in the absence of cyclic AMP but does grow on glucose, fructose and galactose. The growth inability of the mutant *tsC42* at 42° C thus examined was not affected by the sugars mentioned. These results indicate that the defect in the mutant is not associated with functions related to cyclic AMP metabolism. Cyclic AMP acts more generally on the synthesis of a number of inducible enzymes.

Spontaneous reversion frequencies to temperature-resistance were measured in two different clones on complete medium agar plates. Each clone showed the values of 4.6×10^{-7} and 10×10^{-7} , respectively, suggesting a single mutational defect. All twenty revertants so far examined regained the full inducibility of β galactosidase at 42° C. In three of these, the ability to form succinate dehydrogenase and L- α -glycerophosphate dehydrogenase was completely restored at 42° C. This suggests that the loss of the expression of the *lac* operon is also associated with a defect in a function essential for cell growth and the formation of membrane components. The expression of the *lac*-operon may require a newly synthesised membrane component to effectively operate the protein synthesising machine or as a modulator of

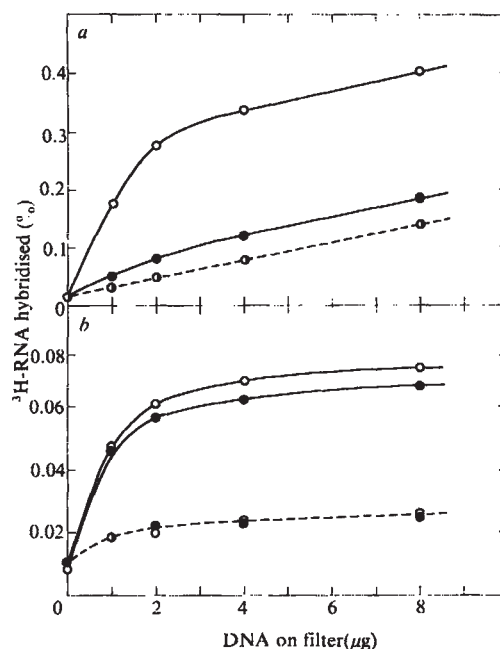


Fig. 2 a, Exponentially growing cells cultured in medium A (ref. 10) divided into four parts, two of them further incubated at the same temperature (30° C) and the others transferred to 42° C. Each group was incubated in the presence (induced) or absence (uninduced) of 0.2 mM IPTG, respectively. b, Exponentially growing cells cultured in Davis's minimal glucose medium with or without tryptophan (100 μ g ml⁻¹) were divided into two parts; one repressed and one derepressed cell were further incubated at 30° C and the others transferred to 42° C. Cells were labelled for 1 min by the addition of 100 μ Ci ³H-uridine (specific activity 26 Ci mmol⁻¹) at 20 min in the case of (a) and 15 min in the case of (b) after the start of the various conditions of incubation. The RNAs from (a) and (b) were extracted with hot phenol equilibrated with a sodium acetate buffer (pH 5.2) containing 0.5% SDS (ref. 13) and hybridised with increasing amounts of $\phi 80$ *plac* DNA or $\phi 80$ *ptrp* DNA fixed on membrane filters according to the method previously described¹³. Ordinate, per cent (added) total radioactivity. ○—○, Induced or derepressed at 30° C; ●—●, induced or derepressed at 42° C; ○---○, uninduced or repressed at 30° C; ●---●, uninduced or repressed at 42° C.

tertiary structure of DNA leading to an active form of the genes (Sankaran and Pogell¹⁸ suggested that the expression of the *lac* operon may be correlated with an alteration in the conformation of DNA.)

Preliminary experiments in which cells were labelled with ¹⁴C-arginine at 30° C and with ³H-arginine at 42° C to distinguish the membrane components formed at a permissive temperature from newly-formed ones at a non-permissive temperature showed that the incorporation of ³H-arginine was restricted to several membrane components separated by SDS polyacrylamide gel electrophoresis.

In vitro studies have revealed that *lac*-DNA, RNA polymerase, cyclic AMP receptor protein, cyclic AMP and *lac*-repressor and inducer are the elements controlling *lac* transcription. The mutational defect in *tsC42* is distinct from those in that the defect is phase-specific, the corresponding function is only operative 20–30 min before cell division and is essential for cell growth and membrane synthesis.

We thank Dr H. Mitsui for discussions, Drs Ozeki and Ohshima for $\phi 80$ *plac* phage, and Drs Imamoto and Segawa for $\phi 80$ and $\phi 80$ *ptrp*-ED-DNA. This work was supported in part by the research Fund of the Ministry of Education of Japan.

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Table 1 Activity of ONPG transport in the mutant *tsC42* and the parent AT713

	ONPG hydrolysed <i>in vivo</i> at: 30° C (μ mol h ⁻¹ ml ⁻¹)	42° C	Relative activity 42° C/30° C
<i>tsC42</i>	0.72	1.74	2.4
AT713	0.81	2.43	3.0

Cells grown in A medium¹⁰ for several generations at 30° C were incubated with 0.2 mM IPTG for 30 min at the same temperature and β -galactoside transport was induced. Cells once washed with A medium¹⁰, containing chloramphenicol (40 μ g ml⁻¹) but without casamino acids, were suspended in the same medium. Transport was measured according to the method of Wilson *et al.*¹⁰.

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Table 1 Inhibition of rosette formation with IgG proteins

Inhibitor		Percentage reacting cells
Experiment 1		
No inhibitor		33.8 ± 2.3
MOPC 21 (parent IgG)	0.58 mg ml ⁻¹	18.0 ± 2.9
IF1 (C _H 3 deletion)	0.58 mg ml ⁻¹	30.9 ± 1.9
IF2 (C _H 1 deletion)	0.58 mg ml ⁻¹	3.3 ± 3.7
Experiment 2		
No inhibitor		34.1 ± 2.5
MOPC 21 (parent IgG)	10 mg ml ⁻¹	0.1 ± 0.1
IF1 (C _H 3 deletion)	10 mg ml ⁻¹	35.9 ± 1.1
Experiment 3		
No inhibitor		43.6
MOPC 21 (parent IgG)	5 mg ml ⁻¹	9.4
IF3 (C _H 3 deletion)	5 mg ml ⁻¹	41.4 ± 1.8
IF1 (C _H 3 deletion)	5 mg ml ⁻¹ (heat aggregated)	43.8 ± 2.9

Pooled lymph node cells from BALB/c mice were used in the experiments. Fc rosette formation was carried out as previously described^{5,6}. For inhibition of Fc rosettes, lymphocytes were pre-treated with inhibitor protein at the concentrations stated for 15 min at 0°C before rosette formation. For one experiment IF1 protein was heat-aggregated at 63°C. The results are the means of more than three replicate preparations when the standard error of the mean is given. Two observations in experiment 3 were the results of counting over 200 cells in a single preparation.

C_H3 domain of IgG as binding site to Fc receptor on mouse lymphocytes

MEMBRANE receptors recognising the Fc portion of immunoglobulin molecules (Fc receptors) are found in many cells of the immune system¹⁻⁴. Fc receptors on lymphocytes are readily detected by a rosette test^{3,5,6} and this reaction is inhibited by pretreatment of the lymphocytes with IgG (ref. 3). IgG proteins lacking almost the entire C_H1 and C_H2 homology regions have been obtained from mutant cell lines of MOPC 21, a plasmacytoma secreting IgG1 (refs 7 and 8) and the extent of the deletions determined (Fig. 1). To identify that part of the IgG molecule which interacts with the Fc receptor, we have tested the ability of these IgG proteins to inhibit Fc rosette formation on murine lymph node cells. We show (Table 1) that an intact C_H3 region is essential for the binding of IgG to Fc receptors on lymph node cells.

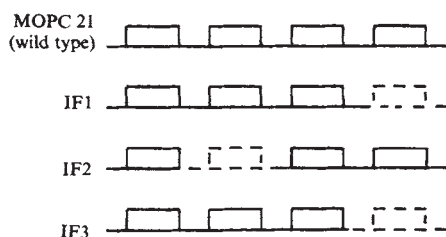


Fig. 1 Structure of the mutant proteins. Dotted lines indicate deletions in the heavy chains, shown diagrammatically, with the four intra-chain disulphide bridges. IF1, IF2 and IF3 were cloned tissue culture lines of the mouse plasmacytoma MOPC 21. IF2 has a deletion in the heavy chain of residues 121 to 214 corresponding to the wild type protein^{8,16}, that is, of almost the entire C_H1 region. IF1 has a deletion of residue 358 onwards¹⁶ and IF3 of residue 342 onwards (K. A. and C. Milstein, unpublished). The secreted protein used in the experiments was purified from the serum of mice bearing the respective tumours, using ammonium sulphate precipitation and DEAE cellulose chromatography. The purity was checked using electrophoresis on cellulose acetate strips, and isoelectric focusing on polyacrylamide gels.

The ability of IF2 protein to inhibit Fc rosette formation shows that the C_H1 region is not the site through which IgG binds to Fc receptors. The failure of IF1 and IF3 proteins to inhibit Fc rosettes shows that an intact C_H3 region is required for the binding of IgG to Fc receptors. Since both IF1 and IF3 possess intact C_H2 regions, this region may not be required for binding to Fc receptors. It is possible, however, that the absence of the C_H3 region in IF1 and IF3 can result in a rearrangement of the C_H2 region, destroying an active site wholly or partly within this region. The inability to detect the C_H3 region of membrane-bound IgG on lymphocytes, with a fluorescent antiglobulin possessing an anti-C_H3 region

specificity, also suggests that it is the C_H3 region which interacts with the lymphocyte membrane⁹.

In an extension of the domain hypothesis, Edelman *et al.*¹⁰ proposed that each of the homology regions of the polypeptide chains of immunoglobulin molecules folds to form a compact domain which has evolved to perform independent functions. Thus the V_H and C_H2 regions of IgG possess the antigen binding and complement fixing sites¹¹, respectively. The binding of IgG to human monocytes¹² and guinea pig macrophages¹³, possibly through a site in the C_H3 region, has been suggested as evidence for the domain hypothesis. The binding of IgG to Fc receptors on K cells¹⁴, neutrophils¹⁴ and a non-secreting plasmacytoma¹⁵ also requires an intact C_H3 region. The C_H3 region of IgG seems therefore to possess the property of binding IgG to Fc receptors, which may be functionally relevant, on many types of cells.

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matters arising

Orogenic zones in central Australia

DUFF and Langworthy¹ present a number of arguments purporting to confound any suggestion of a subduction model² for the Giles Complex–Woodroffe Thrust Zone located along the contact of the Amadeus Basin with the Musgrave Block. During the orogeny associated with this contact, the Petermann Range orogeny³, the north-recumbent nappes developed and thrusting occurred. I have confined my arguments in all instances to this orogenic belt. Any later orogeny, for example, the Alice Springs orogeny, affecting other regions may well be explained otherwise.

this age that is correlated with the tectonic–orogenic episode. If the Petermann Range orogeny is some 1,100–1,150 Myr old, the Arunta and Musgrave Blocks would have moved as a unit from this time onwards, in keeping with the palaeomagnetic evidence.

Duff and Langworthy list features that they claim do not occur in the Petermann Range orogenic belt, thus disproving a subduction model for this region. In this respect, note that acid intrusives, for instance, occur within the orogenic belt³. It has been argued elsewhere that the Giles Complex may well be segments of an ophiolite sequence and it has reasonably been suggested that the extent of represen-

The presence of possible examples of ensialic mobile belts within African and North American cratonic regions⁴ does not preclude the possibility of other orogenic types in specific locations within the Australian craton. It seems to me that a subduction model more than reasonably fits the data available in these areas for the Petermann Range orogeny. Present-day intraplate tectonic compression⁵ does not support the argument for ensialic orogeny unless a correlation can be shown between regions of intraplate compression and regions of thick sedimentation of a specific nature and with igneous activity. This constraint is met by regions of present-day subduction and continental shelf regions and thus these zones can be used as models for past geological events.

In conclusion, in the case of the Petermann Range orogeny the ensialic concept does not explain the following features: the major lithological and structural differences of the basement rocks in contact along the thrust zone the mafic/ultramafic zone; the significance and emplacement of the Giles Complex; the asymmetric nature of nappe distribution and the recumbency of these structures to the north; the spatial and temporal distribution of discrete tectonic elements from south to north. It also seems that the most critical data in the future will be palaeomagnetic and geochronological data relating to the Arunta and Musgrave Blocks specifically.

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Table 1 Radiometric ages for the Petermann Ranges and for an intrusion in the Musgrave Block

Rock types	Age (Myr)	Method*
Granitic gneiss	1,190 (whole rock)	Rb/Sr (3)
	600 (biotite)	
Biotite granite	1,150 (whole rock)	Rb/Sr (3)
	600 (biotite)	
Adamellite	1,123 (whole rock)	Rb/Sr (5)
	1,094 recalculated	
	1,077–1,092	K/Ar (6)

*Numbers in parentheses indicate source reference no.

A fundamental argument presented by Duff and Langworthy relates the time of the orogeny to palaeomagnetic evidence indicating that the Arunta and Musgrave Blocks have moved as a single unit since the late Proterozoic or early Palaeozoic. Even if the palaeomagnetic data⁴ are accepted, a subduction model is in no way contravened, as geochronological evidence clearly indicates that the Petermann Range orogeny occurred about 1,110–1,150 Myr ago. Duff and Langworthy have quoted Forman³ as specifying an age of 600 Myr for this orogeny. Forman gives radiometric ages from specimens collected from the Petermann Ranges and these figures, together with an age given by Wilson and Green⁵ for an adamellite intrusion in the Musgrave Block, are shown in Table 1. The granitic rocks show intrusive contacts with folded quartzites³ in the Petermann Range area.

The question then is whether the whole rock age or the biotite age is taken as dating the orogenic episode. Most authorities now regard biotite ages as excessively low because of the low blocking temperature of this mineral⁷. The biotite age is probably indicative of a late stage of cooling of the crystalline rock and this could be related to uplift and erosion. The whole rock age is generally accepted as the age of initial crystallisation, so it is

tation of certain criteria indicative of subduction, for example, blue-schist facies rocks and calc-alkaline volcanics is very much dependent on the depth of erosion². Erosion would be considerable in Precambrian orogenic belts. Rocks outcropping in the Petermann Range orogenic belt have formed under high pressures and temperatures, that is, at deep crustal levels, so low-temperature and low-pressure rocks are unlikely to be found *in situ*, but this does not mean that such rocks did not form.

Matters arising

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DRS DUFF AND LANGWORTHY REPLY—Davidson^{1,2} believes that a simple plate-subduction model explains certain features of the Musgrave Block, adducing support solely from the alleged similarity of sections through the central Australian shield with 'cartoon' plate-tectonic sections³. He argues^{1,2} that the usual features of plate-edge tectonism—pillow basalts, pelagic sediments, calc-alkali volcanics,

blue schists—are absent because they are above the depth of present erosion. Since that proposition can be neither demonstrated nor refuted his essential thesis amounts to a non-argument. Moreover, the notion of supracrustal erosion of subduction evidence² is difficult to reconcile with the proposed preservation of segments of the ophiolite suite.

He also envisages² a single 1,150 Myr tectonic event, the Petermann Ranges orogeny⁶, during which thrusting, igneous activity and folding, affected basement and cover south of the Amadeus Basin. Within this event, he incorporates the generation of the Woodroffe Thrust as well as the Petermann Ranges nappes in the Dean Quartzite. The whole-rock age of 1,150 Myr used by Davidson² to date the orogeny, however, applies only to a granite unconformably underlying the deformed Dean Quartzite^{4,6}; and it has not been demonstrated that basement thrusting further south was necessarily synchronous with cover deformation or granite intrusion. The Petermann Ranges orogeny therefore probably occurred about 600 Myr ago⁸. As we have indicated⁷, available palaeomagnetic data⁸⁻¹⁰ preclude relative movement between blocks during this period.

The Giles Complex is not, as claimed^{1,2}, a 'segment of an ophiolite suite'. The complex consists of intrusive sheets that contain relatively high pressure assemblages¹¹ suggesting that it was intruded into the lower continental crust¹¹⁻¹³. The Gosses Pile^{1,13} is a layered, ultramafic intrusive sheet that crops out 50 km south of the Woodroffe Thrust and consists of 'orthopyroxenite, websterite, and olivine-orthopyroxenite arranged in a cyclic sequence such as is found in the ultramafic parts of other layered intrusions'¹³. In no way can the Giles Complex be construed as segments of a Proterozoic oceanic crust.

As Davidson¹ himself has previously noted there are no major lithological and structural differences of basement rocks across the Woodroffe Thrust zone.

Geological¹⁴ and recent palaeomagnetic¹⁵ work in certain Precambrian regions has indicated that uniform application of plate-subduction models to such terrains is suspect¹⁵, and we believe that indiscriminant application of such concepts to terrains not fully understood, may serve only to mask the actual tectonic processes.

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Lunar magnetic anomalies and the Cayley Formation

MAGNETIC anomalies may or may not relate to geological units. Strangway *et al.*¹ incorporated geological data in attempting to equate surface anomalies at the Apollo 16 site with those recorded from orbiting Apollo subsatellites, and then correlated the northern plains in the crater Van de Graaff with the Cayley Formation. This correlation is incorrect for three reasons.

First, planar fill in the north end of Van de Graaff is low-albedo mare material with mare ridges, not Cayley-like plains (Fig. 1). Also, the magnetic anomaly near Van de Graaff lies between craters Aitken and Van de Graaff², so that the anomaly could just as easily be ascribed to the acknowledged maria^{3,4} in Aitken. Finally, Strangway *et al.*¹ cited similar crater densities for the Van de Graaff mare and the Cayley Formation at Descartes, thus implying similar ages. Apollo mapping camera photographs show, however, that the maria in Aitken and Van de Graaff (NASA photographs AS17-1385 and AS15-0076) have similar primary crater densities (Fig. 1)

whereas the Cayley plains seem to have significantly more 0.5-1.0 km craters (NASA photograph AS16-0162) and are therefore older.

Orbital magnetic data do not substantiate the correlation of Cayley-like plains with any particular magnetic signature. The other significant magnetic anomalies on the centre far side are not near or over large areas of light plains but instead occur over a wide variety of terrain²; there is no significant anomaly over Cayley Formation plains on the near side of the Moon. If flows of hot breccia from basins were the main cause of lunar magnetic anomalies⁵, then the basin Hertzprung should show a large anomaly because it is extensively filled with Orientale ejecta. Early reduction of Apollo 15 data⁶ showed a weak anomaly, but this vanished after Apollo 15 and 16 data were integrated (L. R. Sharp and P. J. Coleman, unpublished data).

The outstanding magnetic anomaly between Aitken and Van de Graaff (Fig. 1) correlates with no unique geological feature. The same type of geology occurs elsewhere without magnetic signatures. Therefore, the best explanation for the anomaly remains a subsurface source².

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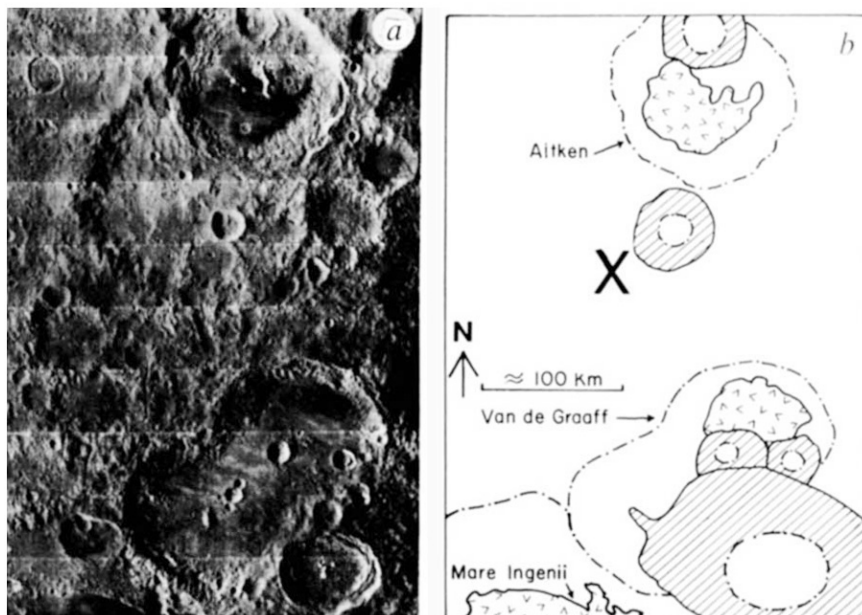
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Fig. 1 *a*, NASA photograph of the Aitken-Van de Graaff area, showing the low-albedo maria in these two craters and in Mare Ingenii (Orbiter II, moderate 33); *b*, sketch map shows approximate centre of the magnetic anomaly (X). —, Contacts; - - -, crater rim crests; lined pattern, post-mare crater ejecta; 'v' pattern, mare rocks.



Shadow of the head of Comet Kohoutek

HOPKINSON, Elsworth, and James¹ have reported the detection of a straight dark streak running away from the head of Comet Kohoutek (1973f) for a distance of about 2.5° . They conjectured that the streak could be the shadow of the comet's head as seen in the dust tail of the comet. Their observations were made on January 9, 10, 11, and 12, 1974 at about 2200 UT.

We obtained photographic observations of Comet Kohoutek at the Joint Observatory for Cometary Research (JOCR) near Socorro, New Mexico. We have observations which overlap in time with those obtained by Hopkinson, Elsworth, and James; a sample taken on January 11, 1974 UT is shown (Fig. 1). Other JOCR plates have been discussed by Hyder *et al.*². Our primary plate emulsion was IIA-O, but we also have plates with IIA-E and IIA-F emulsions. Colour photographs (Ektacolor-L) were also taken.

We feel that the shadow, if real, should be found on our plates, but we have found nothing that we would interpret as the shadow of the head extending 2.5° (5.4×10^6 km) in the antisolar direction. At the time of the exposure shown in Fig. 1, the prolonged radius vector was very close to the axis of the plasma tail as projected on the plane of the sky. We have found two features which conceivably could be responsible for the earlier observations¹. First, a linear dark area was found on a IIA-E plate taken shortly before the IIA-O plate shown in Fig. 1. This dark streak is in the head, on the side away from the dust tail. Unfortunately, it is neither long enough nor in the correct position to concur with the observations of Hopkinson, Elsworth, and James. In addition, inspection of the IIA-O plate shows clearly that this dark area is just the space between tail rays of the plasma tail. Similar dark streaks pointing toward the nucleus are found on the other side of the head (Fig. 1), but they are not as prominent because the

scattered solar radiation from the dust tail tends to fill in the dark spaces. Second, on low resolution photographs the space between the plasma and dust tails could give the appearance of a dark streak.

Whatever the ultimate explanation, we note that our plate scale is $300'' \text{ mm}^{-1}$, and we estimate that our resolution (which we consider to be caused by plate scale, image quality and emulsion characteristics) is approximately 10 times greater. The difference in resolution is probably the root of the discrepancy.

Finally, in terms of cometary physics, we believe that observation of the shadow is unlikely for two reasons. First, our colour photographs indicate a low dust content and a relatively weak dust tail as compared with Comet Bennett (1961i). Thus, it is unlikely that observable dust would extend out to 5.4×10^6 km (2.5°) except near the axis of the dust tail. Second, the dust tail (presumably composed of micron sized dust) curves away from the antisolar direction. At 5.4×10^6 km from the nucleus, the axis of the dust tail lies well away from the antisolar direction.

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¹ Hopkinson, G. R., Elsworth, Y., and James, J. F., *Nature*, **249**, 233 (1974).

² Hyder, C. L., Brandt, J. C., and Roosen, R. G., *Icarus* (in the press).

Platelet monoamine oxidase in schizophrenics

MURPHY and Wyatt reported reduced monoamine oxidase (MAO) activity in blood platelets from 33 chronic schizophrenic patients¹ and 13 monozygotic twin pairs² compared with normal controls. They suggested that reduced platelet MAO activity may provide a genetic marker for vulnerability to schizophrenia. We predicted lower MAO activity might sensitise the

schizophrenic patient to the induction of symptoms by tricyclic antidepressants³.

We have completed a study of 24 anergic, depressed schizophrenic outpatients. Patients were free of psychoactive medication for 2 weeks and then blindly treated with either chlorpromazine (Thorazine, SKF) 100–1,200 mg daily and imipramine (Tofranil-Geigy) 150 mg daily or thiothixene (Navane-Roerig) 5–60 mg daily and placebo. Platelet MAO activity was measured following the drug-free period and weekly for 4 weeks on medication, using the method of Wyatt and Murphy^{1,2} with ^{14}C tryptamine (1.6×10^{-5} M final concentration) as substrate. Blood samples from eight alcoholic patients and seven staff volunteers served as controls.

We found no differences in platelet MAO activity in the schizophrenic patients compared with alcoholics and volunteers. On the basis of MAO platelet activity (mean $\pm$ standard deviation in nmol product formed per h per mg protein) individuals from all three groups could be assigned to either a low (1.62 ± 0.34) or high (3.09 ± 0.53) MAO group, with no overlap. Our low MAO group contained 13 schizophrenic patients, four alcoholics and three staff. The high MAO group consisted of 11 patients, four alcoholics and four staff. Discriminant function analysis distinguished high from low MAO level patients on the basis of hyperactivity (Katz adjustment scale factor), sleep disturbance and apathy (Hamilton depression scale factor). No significant differences in primary schizophrenic symptoms existed between patients in the low and high groups at baseline. Preliminary data analysis of 4-week improvement indicates low MAO group patients may do significantly less well on the chlorpromazine-imipramine combination. These findings are under investigation in schizophrenic inpatients.

We have been unable to replicate the reduced blood platelet MAO findings in schizophrenic patients as reported in *Nature* by Wyatt and Murphy¹. Individuals have either low or high MAO activity independent of diagnosis. Activity remains stable over at least five week periods. Platelet MAO activity may influence the schizophrenic patient's response to tricyclic medication.

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DRS MURPHY AND WYATT REPLY—
Shaskan and Becker's interesting data may be clarified by other recent MAO

Fig. 1 Comet Kohoutek (1973f) on January 11, 1974 UT. This 2 min exposure was taken between 2:59:20 and 3:01:20 UT. The field shown is approximately $3^\circ \times 4^\circ$.



studies. In addition to our reports^{1,2}, Nies *et al.*⁴ observed significantly reduced MAO activity in schizophrenics, as did Meltzer and Stahl⁵, who also noted differences in substrate specificity differentiating chronic from acute patients. Our recent study of acute schizophrenics revealed normal MAO activity⁶, while a replication study of chronic schizophrenics again documented reduced MAO activity unexplained by differences in thyroid or sex steroid hormones, iron metabolism or drug treatment (in preparation). This distinction between chronic and acute schizophrenics is interesting in view of studies of adopted-away children of schizophrenics indicating that chronic and acute schizophrenia may be genetically distinct disorders⁷.

While the non-overlapping low-high distribution of MAO activities observed by Shaskan and Becker in 24 schizophrenics might represent a split between chronic and acute patients, the bimodal distribution in eight alcoholic and seven staff controls suggests the possibility of sampling inequities or technical differences in this very small sample. A typical unimodal distribution pattern for platelet MAO was observed in 167 normals⁸. Whether alcoholics should be included as 'controls' is problematic, as alcoholism has been linked to affective disorders, and patients with bipolar affective disorder have reduced MAO activity⁹.

We have also found platelet MAO activity to be a generally stable characteristic of individuals. We continue to view MAO differences as possibly related to vulnerability issues (which might, for example, result in chronicity) rather than to overt psychiatric disorders themselves^{2,8}. Shaskan and Becker's observation that low MAO patients may be relatively treatment-resistant is congruent with this concept. In studying non-hospitalised, drug-responsive patients, however, Shaskan and Becker have not replicated the conditions of our first study of chronic schizophrenics, but instead may have accomplished a combination study of schizophrenic subgroups. We look forward to further examination by them and others of the relationships between MAO activity, behaviour and behavioural disorders.

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⁸ Murphy, D. L., Belmaker, R., and Wyatt, R. J., *J. Psychiat. Res.* (in the press).

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Chloride ions cancel out inhibition of β -galactosidase activity by acid mucopolysaccharides

A SERIES of short reports on the effect of glycosaminoglycans on β galactosidase have been published¹⁻³. Kint³ has shown that cetylpyridinium chloride or albumin can partially overcome the inhibition of β galactosidase by the glycosaminoglycans accumulated in autopsy liver homogenates from mucopolysaccharidoses patients. We report that this inhibition is completely cancelled out by a pure physiological agent, that is, chloride ions, required for enzyme activity⁴⁻⁶.

The effect of various glycosaminoglycans and synthetic sulphated polymers on acid β -galactosidase activity is summarised in Table 1, which shows that hyaluronic acid was not inhibitory whereas all the sulphated polymers were inhibitory to varying extents. Chondroitin sulphate A and keratan sulphate were only mildly inhibitory but the synthetic sulphates were very strongly so. Chloride ions cancelled out all inhibition completely. The lowest concentration of chloride ions which prevented inhibition resulting from 83 μ g ml⁻¹ heparitin sulphate was about 1.6 meq.

Other salient observations were as follows. (1) Chloride ions prevented inhibition if added simultaneously with inhibitor in the incubation mixture. Preincubation of enzyme with inhibitor at 37° C led to irreversible inactivation after 5–10 min and subsequent addition of chloride ions did not restore enzyme activity. (2) Inactivation of enzyme by glycosaminoglycans required heat and acidic pH (below 4.5). A mixture of enzyme and inhibitor maintained at 0° C did not result in inactivation or complex formation. The same mixture

Table 1 Effect of glycosaminoglycans on acid β galactosidase activity in the presence or absence of chloride ions

Addition	Enzyme activity (%)	
	–Chloride	+Chloride
None	100	138
Hyaluronic acid	116	139
Chondroitin sulphate A	73	135
Keratan sulphate	73	134
Dermatan sulphate	51	126
Heparitin sulphate	20	133
Dextran sulphate	7	88
Polyvinyl sulphate	5	80

A 15% homogenate of liver in distilled water was diluted 1:20 with water or 0.2 M NaCl and assayed for β galactosidase activity using 4-methylumbelliferyl- β -galactoside as substrate⁶. Incubations were carried out for 15 min at 37° C. The final concentration of chloride ions in the assay mixture was 33.3 meq., that of glycosaminoglycans/sulphated polymers 83 μ g ml⁻¹.

Table 2 Effect of chloride ions on β -galactosidase activity in mucopolysaccharidoses patients and in controls

Liver homogenates	Enzyme activity	
	Chloride	–Chloride
Control (1)	160	180
Control (2)	264	309
Hurler (MP-I)	7	22
Hunter (MP-II)	5	30
Sanfilippo (MP-III)	15	58

Conditions are as in Table 1. Enzyme activity is expressed as nmol substrate hydrolysed per h per mg protein.

on starch gel electrophoresis at 4° C (ref. 6) gave an isoenzyme pattern indistinguishable from controls.

The effect of chloride ions on β -galactosidase activity in liver homogenates from mucopolysaccharidoses patients and from controls is shown in Table 2. The results indicate that the apparent restoration in enzyme activity in the mucopolysaccharidoses patients was incomplete, suggesting that irreversible enzyme inactivation had occurred. Further inactivation of enzyme was prevented, however, by the presence of chloride during incubation, as evidenced by the three to fourfold increase in activity in all patients when chloride was included in the assay mixture.

It is now clearly established that the β -galactosidase abnormality in the mucopolysaccharidoses patients⁷ is not the primary genetic defect¹⁻³. The casual relationship between the reduction/inhibition of β -galactosidase activity and the clinical manifestations of the diseases is not known. Therapy along the lines of 'restoration' of β -galactosidase activity should be considered only when the above correlation is clearly demonstrated. If such therapy is required, chloride ions (administered for example as a saline infusion) should be chosen as they are relatively innocuous compared to the alternatives suggested³.

This work was done in the laboratory of Professor John S. O'Brien, Department of Neurosciences, University of California, San Diego. We thank Professor O'Brien for liver autopsy samples.

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¹ Kint, J. A., Dacremont, G., Carton, D., Orye, E., and Hooft, C., *Science*, **181**, 352–354 (1973).

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reviews

THIS is an important book*. It is important because in it a man most competent to do so, sets out society's most important problem: self-control and regulation. It is a problem which has become increasingly urgent over the last decade. In this book Stafford Beer, perhaps the world's leading exponent of cybernetics in action, sets out the problem again and again in a series of addresses which he has given over the years: a presidential address

to the Operational Research Society; a chairman's address to the International Cybernetics Congress; a presentation to the committee on Science and Astronautics of the US Congress; a key-note address to the Conference on Ecological Systems; a presidential address to the Society for General Systems Research; and many others. In each address the message is repeated, amplified and extended. Because the talks were given to a variety of audiences and because Stafford Beer writes in a readable style they can be understood by the non-specialist. Far from being boring the repetition serves to drive home the message just as walking round and round a building gives a lasting impression. The separate addresses are welded into a whole by a thesis running through the book (blue pages) and linked by a personal narrative (yellow pages) and a metasystemic overview (gold pages). On these

other than white pages the script is broken down into what seems to be blank verse but what is really a grouping of words and phrases to make them more noticeable. This novel method is certainly effective even though it is irritating at times. It gives the effect of shouting, but that may only be until we get used to it.

Novel as they are the mechanics of the book and indeed the powerful highly personalised style of the author

are less important than the message. Stated simply the message is as follows: man must learn to control the increasing complexity of his world; there is a way of doing this known as cybernetics and general system theory; man's thinking and institutions make it impossible for him to adopt this method; result: loss of control and disaster.

"Thanks to the growth of complexity, which is very much a function of the

native courses ahead. The first is that nothing is done: we carry on as we are. Then the component of future trajectories that is in principle adaptive will, I predict, fail to adapt—because it is frustrated by an organisational straitjacket. And the component that is inexorable will, I predict, take society on to structural collapse or to its overthrow by revolution. The second alternative is that we should create a meta-system to handle the metathreat."

So the problem is clear: increasing complexity. And the solution is clear: the applied science of control and regulation tooled by computers. What then is the problem? Quite simply that the solution cannot be applied. That is really what the book is about. A plea, even an evangelical plea, for society to use the solution available to it. What is the alternative system that society seems to prefer? It is the classic 'muddle-through', a sort of creeping evolution that consists of doing nothing structural but reacting to the immediate situation and relying on cataclysmic upheavals for structural change. As Stafford Beer points out, society is so complex that reacting only to the immediate is quite ineffective and also overlooks the different lag and lead times in the system (witness our economic nonsenses). Furthermore, cataclysmic upheaval is likely to be catastrophic. Finally, the wrong decisions (as in nuclear affairs)

can lead to irreversible situations.

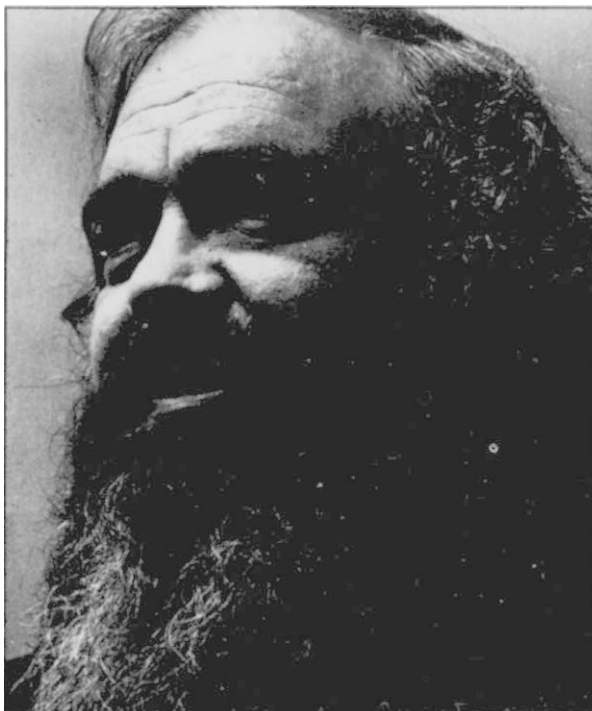
Why, then, is society so reluctant to adopt the system approach so clearly set out for it by Stafford Beer and his peers? The author thinks that we need a new kind of man: man the controller rather than man the maker.

"The difficulty is how to replace *Homo faber* with a new kind of man. He will not be man the maker any longer. He will be man the steersman—of large complex, interactive systems. I call him *Homo gubernator*."

Stafford Beer blames also our antiquated thinking habits and especially their stultifying dominance by the

New order for society

Edward de Bono



Stafford Beer

growth in data handling capacity and of the information explosion, society has outgrown the dynamic regulating capacity of its own hallowed structure."

"Handling complexity seems to be the major problem of the age, in the way that handling material substance offered challenge to our forefathers. Computers are the tools we have to use and their effective use must be directed by a science competent to handle the organisation of large, complex, probabilistic systems. This is the science of cybernetics, the science of communication and control."

"There seem to be only two alter-

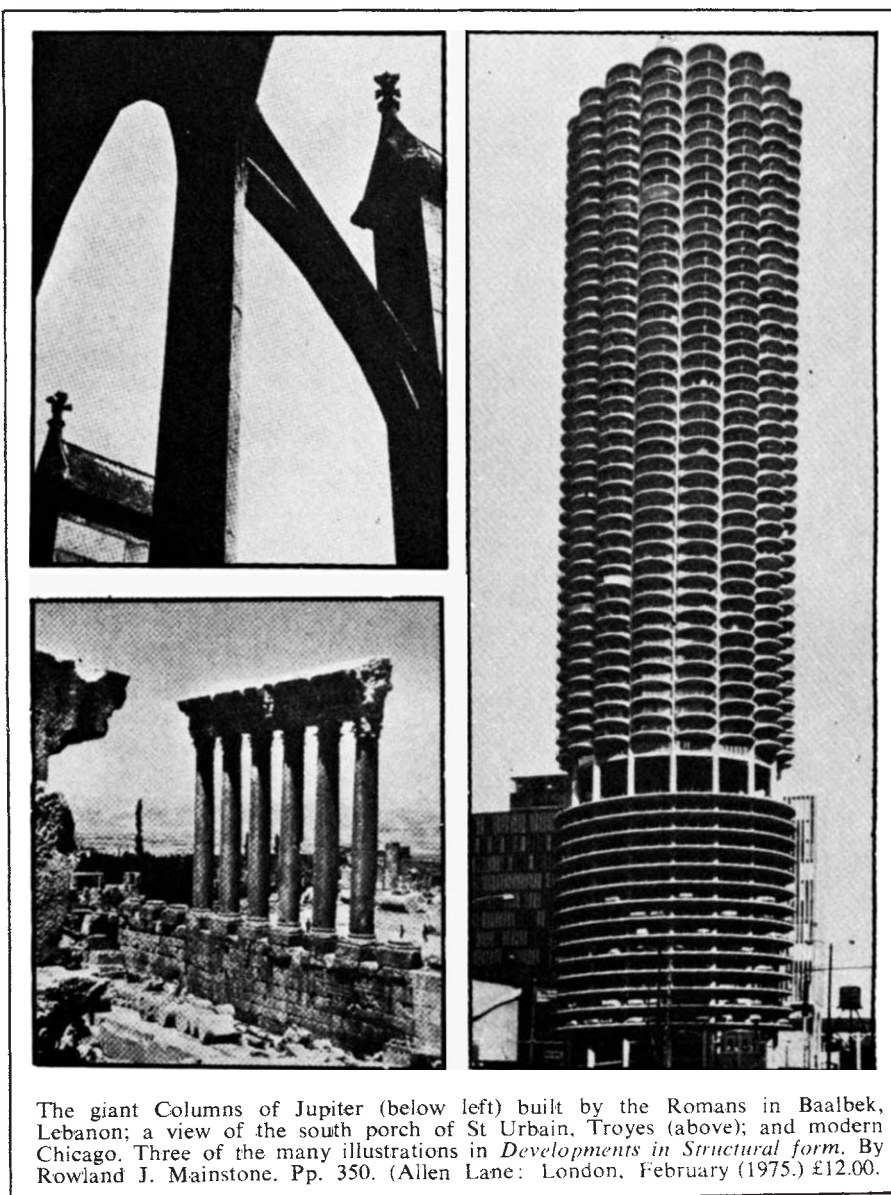
**Platform for Change*. By Stafford Beer. (Wiley: London and New York, January 1975.) £5.00.

Greek tradition of analysis. He points out that the Greeks had no concept of system: "As far as I can tell, the Greeks—even the greatest of the Greeks, Aristotle—had not the faintest glimmer of understanding here." I am very much inclined to agree with him. We forget just how much our academic and thinking traditions are dominated by the particulate analysis and logical reconstructions we have inherited from the Greeks. Our ordinary government is based entirely on such idioms: analyse, define, judge, choose, act. In one of the sections the author lets fly at the media in general and the BBC in particular for castrating any attempt by science to put over system concepts or science in general. This is a little unfair but there is no doubt that the media allocation to fundamental concepts such as those outlined by the author is infinitesimal (on the basis that no one would be interested). "... a colossal effort of will is required to dislodge the trammelines of thought which have by now set in concrete."

Stafford Beer makes great use of the concept of metalanguage and meta-systems. A metalanguage is a language equipped to decide what is otherwise undecidable in lower order languages, and a metasystem does likewise: "In short I am making a straight appeal to the logical transcendence at the meta-systemic level of limitations imposed on the lower-order logics by Gödel's Theorem". At one point he attributes much of the disorder in society to the loss of the metasystems imposed by religious, moral and legal frameworks. He also tries to revive the idea of 'eudemony' (based on one of the Greek words for happiness) roughly meaning a "complicated image of well-being", suggesting it as the new measuring quality, or metric, of human systems.

The message is well put together and utterly convincing. Opponents will, of course, ask why those occasions when system planning was given its head were not great instances of its effectiveness. It is said the Vietnam war was organised on this basis. Stafford Beer himself was a chief adviser to Allende's Chile. The explanation offered by the author is that in no case were the systems able to operate effectively. Chile, he claims, was the victim of an international assassination. All that is true but should a system not be designed to take account of the environment in which it is supposed to behave?

We come now, I think, to the weakness of the argument. Systems can indeed optimise the control and performance of the units or concepts embodied in them. But what are those concepts to be? We can optimise the flow of 10-ton trucks along a road or, indeed, transport between two points but what of a change in concept that does away



The giant Columns of Jupiter (below left) built by the Romans in Baalbek, Lebanon; a view of the south porch of St Urbain, Troyes (above); and modern Chicago. Three of the many illustrations in *Developments in Structural form*. By Rowland J. Mainstone. Pp. 350. (Allen Lane: London, February 1975.) £12.00.

with the need for transport between those two points? There is, to my mind, a large and very significant element lacking in systems theory. It is the lack of this element which makes people suspicious of handing over their future to a group of cyberneticians. Stafford Beer himself asks why people should trust the cyberneticians: "Who are we to say we know best and everyone else is wrong? . . . It is that we are responsible. We are not responsible because we have been elected to govern affairs; we are responsible because cybernetics, that science of effective organisation, is our profession."

I do not think that will do. That is a blatant cry for a priestly cast who alone know the rites of worship and control. We must admit that societies run by priests have been most effective (Aztecs, Incas, and so on) but we also know that they can get their gods wrong with bloody consequences for everyone. There is no doubt that the author is not seeking power of a cen-

tralised sort and he makes a point of saying that he wants the same sort of regulation that keeps the body temperature at 98.4° F by a method of complex interrelating feedback systems rather than through centralised dictat.

I think that Stafford Beer makes a very good case for systems control and for the doom that awaits society if it takes no heed. But there is a great deal still to be done by the systems people who must learn to design systems that will be accepted and used by society on a basis other than exhortation and threat. Selling a system is also part of the system.

"... The Second Law of Thermodynamics has two forms. One is concerned with the pressure to even out energy: that is the form that belongs to our stereotype conception of the universe. It betokens death. The other form is about information content which leads to greater organisation and increasing complexity. That form betokens life." □

DNA Synthesis. Arthur Kornberg. Pp. 399. (Freeman: San Francisco, 1974.) \$18.00.

THIS is an updated and expanded version of Arthur Kornberg's earlier book, *Enzymatic Synthesis of DNA*. That was published in 1962 and since then, the field of DNA biochemistry has progressed enormously, but has become, in many ways, more and more complex. So this new book is an attempt by a protagonist of DNA replication to provide an overall review of the biochemistry (as against physiology) of DNA synthesis.

The book has been split into natural chapters, each concentrating on a specific area of interest. The first, on the structure and function of DNA, is a good introduction to the basic physical properties of DNA, and the second is an excellent simplified presentation of nucleotide biosynthesis. It may, however, be said that the section on thymine and thymidine utilisation is perhaps a little brief, especially when one considers the extensive use to which these precursors have been put. A whole chapter is spent discussing *E. coli* DNA polymerase I. (The author's laboratory has, in an enzymological *tour de force* spanning some 15 years, elucidated the detailed enzymology of this enzyme. That work has formed the basis for the characterisation of all other DNA polymerases.) Succeeding chapters describe in turn the two other *E. coli* DNA polymerases, enzymes from other bacteria and bacteriophage-induced polymerases and then the relatively murky field of eukaryote and virus induced DNA polymerases. Later chapters also describe the basic biochemical properties of nucleases and *E. coli* RNA polymerase, although the former is dealt with in a rather cursory manner. That is rather surprising considering the reiterated and necessary emphasis on these enzymes throughout the book. The last chapter addresses itself to nucleotide sequence determination and gene synthesis and manipulation, and considers the social and moral aspects of 'genetic engineering'.

All of that, together with the section on polynucleotide ligase represent an excellent summary of the current (early 1974) knowledge of the biochemistry of nucleic acid enzymes. It is in the realm of DNA replication, however, where the book is more subject to criticism. These chapters present not only the known facts but also the author's personal interpretation of DNA replication and it is not always clear what is generally accepted by workers in the field and what is still under dispute. For example, it is by no means proven that RNA always initiates DNA synthesis. Continued re-

search and the test of time will confirm or disprove Kornberg's thoughts. If they are disproved the book may, because of factual rather than speculative errors, age rather faster than it otherwise ought.

To present an overview of DNA synthesis in such detail in only 400 pages is in itself a remarkable achievement and the result is commendable. Despite a few shortcomings, some of which I have alluded to, the book is particularly welcome to workers in DNA replication in providing a concise and up-to-date compendium of nucleic acid enzymology. In addition, the book has an extremely useful comprehensive bibliography that not only follows the text but is also cross-referenced to both authors and subjects. The style of presentation and the good organisation makes for comprehensible (if occasionally encyclopaedic) reading and as such should also be of value to those with only a passive interest in DNA synthesis.

Ian J. Molineux

Nucleic acids

Physical Chemistry of Nucleic Acids.

By Victor A. Bloomfield, Donald Crothers and J. R. Tinoco. Pp. x+517. (Harper and Row; New York and London, 1974.) £12.50.

SPECIALISED monographs are aligned between two extremes: that of the scholarly work with a personal flavour, which serves to lever the jaded research worker from his rut so that he may view the surrounding countryside from a new vantage, and that of the encyclopaedic work that provides little but map references to where he may want to go. This book, tends to the latter extreme and is founded on a necessary compromise.

To cover in a manner comprehensible to the novice the theory of all the experimental methods mentioned, to discuss all the ramifications of their interpretation, and to present even the salient results available in the vast literature on the physical chemistry of nucleic acids and their components, would require many, many volumes. On the other hand, to present only the experimental findings would be incomprehensible to all but the specialist—and who is a specialist in all aspects of this subject? As a compromise the reader is referred to standard texts for standard theories, and only those not easily found are discussed in detail.

The opening chapters deal with the properties of the constituent bases, nucleosides and nucleotides and with attempts to account for their spectro-

scopic and thermodynamic properties in quantum mechanical terms. The book progresses to a consideration of single stranded oligomers and polymers before coming to grips with various aspects of double helical complexes. A final chapter deals with transfer RNA. Despite this orderly progression, the preoccupations of the authors are sometimes in evidence and readers fascinated by the intricacies of the interpretation of the properties of RNA may be disappointed by the emphasis given to double stranded DNA. The absorption, rotation and scattering of radiation by nucleic acids are well covered though because of the compromise on which the book is founded some fierce quantum mechanical formulae occur with but terse discussion of their meaning, in a way that may well deter the novice. Similar strictures apply to parts of the discussions on hydrodynamic properties and other topics. So encyclopaedic is the range of topics covered that the few sins of omissions may be excused: there is scant mention of electrophoretic properties even though electrophoresis in polyacrylamide gels is now a favourite tool of the nucleic acid investigator; similarly there is no mention of photochemical matters.

The reader will require a background knowledge of physical chemistry, though the level of mathematical expertise required should be well within the range of all likely to want to read this book. Specialists, however, may cavil at the treatment of some topics: thus the section on polymer statistics makes no mention of the rotational isomerism theory; perhaps that is because it is at this point that the theory becomes intricate and not suitable for inclusion in a general text of this sort. Similarly, the treatment of helix coil transitions, though one of the few available at this level, is elementary, and the notion of 'partition function' barely mentioned, and at that in awe inspiring italics. These simplifications are in jarring contrast to some of the quantum mechanical formulations which occur elsewhere.

Despite these criticisms this is a very useful book indeed and is the only volume of this scope that has been published in English. The text is clear, and only occasionally does the numbering of equations and figures go astray. Although the references do not go beyond about 1970, I read the book with much profit and would expect both research students and experienced workers to do likewise. I may not remember all that I have read but at least I now know where to find it all. It is worth the price, high as it is, for that reason alone.

E. G. Richards

Keeping healthy . . .

Viral Immunodiagnosis. Edited by Eduard Kurstak and Richard Morisset. Pp. xiv+334. (Academic: New York and London, October 1974.) \$39.50; £18.95.

THE current lack of specific treatment for most viruses has discouraged the demand for rapid and accurate diagnosis of viral infections. Although precise diagnosis is essential from the epidemiological point of view, the results are generally obtained too late to be of practical value for the immediate care of the patient. Nevertheless, the rapid identification of viruses is yielding important information on the role of viruses in infections of the foetus and the newborn; on the prevention of transmission of hepatitis B by blood transfusion and the spread of this infection by other routes; for the diagnosis of acute and chronic infections of the nervous system; and on respiratory infections, rabies, the herpes group of viruses and others. Similarly, the availability of rapid, specific diagnostic methods is playing a major role in the experimental investigation of a variety of viruses, including the tumour viruses.

The labelling of antibodies with enzymes offers a new technique for application in diagnostic virology. The immunoperoxidase method is a specific, rapid and relatively simple technical procedure which allows the localisation by light microscopy and by electron microscopy of viral antigens present in specimens collected directly from a lesion or from infected tissue cultures. Immunofluorescence and immunoferritin are other methods which provide rapid and specific diagnosis. The identification of viral antigens and antibodies by immune electron microscopy has been used successfully not only for

the investigation of immune complex formation and the serotyping of viruses but also for the detection of viral agents which cannot be easily cultured and for determining antigenic relationships of newly discovered viruses.

The practical procedures for the preparation of enzyme labelled antibodies, for the conjugation of fluorescein, ferritin and cytochrome *c* and the application of these techniques are described in detail in this book in a series of authoritative articles by a number of distinguished contributors. Immunodiagnostic techniques offer new methods for the rapid identification of many viruses and these techniques can be readily applied in hospital laboratories as well as in research laboratories. This book thus fulfills an important need by providing a lucid and a reasonably comprehensive guide, drawing attention to current findings and pointing to new directions of research for the clinical diagnosis of viral infections. An example of future prospects is the technique of cytohybridisation which, although not immunological in nature, offers the advantages of relative rapidity and ease and the reliability with which viral genome can be detected whether or not infectious virus is present. The preparation of specific probes requires a relatively sophisticated laboratory but no doubt such reagents will become available commercially in due course.

The book is well written, with a text that is lavishly illustrated and practical instructions that are explicit and complete, and I therefore wholeheartedly recommend it as essential reading for microbiologists and immunologists. It is a pity that in these days of economic stringency the high price may well keep this useful and stimulating volume away from the bench of many laboratories, where it should clearly be readily available.

Arie J. Zuckerman

Parasites in the Immunized Host: Ciba Foundation Symposium. Pp. viii+280. (Elsevier/Excerpta Medical: London, Amsterdam, New York, 1974.) \$16.20.

UNLIKE the majority of bacterial and viral infections, parasites are rarely eliminated totally from their hosts by the action of immunity. Chronic parasitic infections are the outcome of a delicate balance between the immunological forces of the host on the one hand and the circumvention of these forces by the parasite on the other. The purpose of the Ciba Symposium held at the Ciba Foundation in London from November 13 to 15, 1973 was to discuss the various mechanisms by which parasites are able to evade the immune responses of the host; twenty six eminent specialists in the fields of immunology, parasitology and tropical medicine took part.

There are probably several distinctive ways in which parasites survive in the immunised host. Five mechanisms were examined in detail by experts in those particular areas of research. Antigenic variation, which is thought to be a major factor in the survival of parasitic protozoa, was discussed, particularly in plasmodia and trypanosomes. From the analogy provided by the extensive studies on *Paramecium* it seems likely that a switch in gene activity is the mechanism. A form of variation has been described in *Nippostrongylus* but the mechanism in helminths may be different from that in protozoa.

Certain parasites seem to survive by modifying the immunological responsiveness of their hosts; there are several ways in which this could happen. For example, T-cell responsiveness is dampened in lepromatous leprosy and diffuse cutaneous leishmaniasis and antigenic competition is a possible reason for the poor immunological responses in patients with malaria. Antigens of the host-type, which are related to the blood group substances and appear on the surface of schistosomes are thought to protect this particular parasite from the action of antibodies. Many parasites release soluble antigens and it is possible that these substances facilitate the survival of the parasite by such means as inducing tolerance, stimulating suppressor T cells or blocking cytotoxic mechanisms. The ability of certain parasites to survive within macrophages is, perhaps, the most remarkable example of immune evasion. In some cases this may be because the lysosomes are prevented from fusing with the phagosomes containing the organism, but that is not always the explanation.

Each paper presents an excellent review of up to date knowledge of one aspect of this subject and is followed by a full transcript of the discussion it

Ultrasonic Imaging and Holography Medical, Sonar and Optical Applications. Edited by G. W. Stroke, W. E. Kock, Y. Kikuchi, J. Tsujiuchi. Pp. xi+642. (Plenum: New York and London, 1974.) n.p.

THIS is a fascinating book for all who are interested in remote sensing with non-optical waves. A distinguished group of authors, including Denis Gabor, have contributed to this volume. It comprises a complete set of papers on the subject of holographic imaging and information processing, which were presented at the United States-Japan Science Cooperation Seminar in Hawaii, in January 1973. Most of the 20 papers are concerned with the imaging of human tissue, using ultrasound.

The remaining papers report recent developments in acoustic microscopy and optical signal processing of acoustic images. It is clear from this book that ultrasonic imaging is emerging as a particularly useful technique for application in the medical field, as it is a powerful aid to non-invasive early diagnosis of cancer. The book is therefore to be especially recommended to medical physicists, radiographers and clinicians who wish to be aware of the recent developments in this field.

The diagrams and photographs, so essential to the presentation of this subject, are generally quite good although they have not been standardised by the editors. But that is to be expected in what are really conference proceedings.

A. P. Anderson

provoked. Because of the contributions made by immunologists—unfamiliar with, but nevertheless fascinated by parasitology—the discussions are particularly valuable to those more familiar with the parasite systems. It is only recently that we have come to appreciate the ability of parasites to evade immunity and it is not surprising that more questions were posed than answered at this symposium. It is suggested in the introduction, however, that within the next 20 years we shall have successful immunoprophylaxis of many parasite diseases. Immunologists searching for more practical areas of research would be well advised to read this book.

S. R. Smithers

Proceedings of the 6th Berkeley Symposium on Mathematical Statistics and Probability. Vol. 4: *Biology and Health*. Edited by L. M. Le Cam, Jerzy Neyman, and Elizabeth L. Scott. Pp. xvi+353. (University of California Press: Berkeley, Los Angeles and London, 1972.) £11.50.

THE Proceedings of the Berkeley Symposia are too well-known to require much introduction. Volume IV (Biology and Health) of the Sixth Symposium contains 25 papers divided into five sections covering "Clinical Trials and Sequential Procedures", "Population studies and branching processes", "Biostatistics", "Cellular Phenomena and Carcinogenesis" and "Psychological Aspects of Observational Studies".

Although the papers on clinical trials and sequential methods were interesting, the important distinction between the two objectives of clinical trials (decision making and investigation of the natural history of diseases) was not made. The extent to which the decision orientated, sequential design can satisfy the second objective is arguable and it would have been valuable to have some discussion of that point. Another basic problem of the application of sequential designs in clinical trials is their dependence on a single response variable both for the stopping rule and the final decision. In many trials situations, however, particularly with chronic diseases, there is no single response variable of major interest. Instead, a battery of responses, of more or less equal status, are noted. To use a sequential design in this situation implies that one response variable must be chosen subjectively as being that on which the sequential procedure depends. Some discussion of these and other difficulties would have given a better balance to this section.

Although most of the papers give interesting mathematical treatment to realistic biological problems, the relevance of some of the papers to *Biology and Health* is not obvious, a pity in a volume with this title. J. A. Anderson

... growing old

Intrinsic Mutagenesis. A Genetic Approach to Ageing. By Sir MacFarlane Burnet. Pp. ix + 244. (MTP Medical and Technical Publishing: Lancaster, 1974.) £6.75.

OVER the years since gerontology became a recognisable discipline, there seem to have been almost as many theories of ageing as there have been workers in the field. This top heavy development, and the relative dearth of contributions from the more renowned workers in those research fields which have a bearing on the ageing process, may well have been responsible for the very tardy award of any accolade of respectability to gerontology. Recently, however, writers such as Sir MacFarlane Burnet have entered the field and are doing much to elevate age research to its proper place among the biological sciences. This book cannot but continue this highly desirable process.

There are two broad theories of ageing, both of which have been under continuous discussion over the past decade. On the one hand are the theories that ascribe programmed origins to the ageing process; on the other hand, the theories that ascribe stochastic or random origins to that process. Sir MacFarlane considers both theories, and develops a new concept in which parts of both have a role to play. His present theory is based on the belief that a certain degree of mutation is required to provide the optimal introduction of the new information necessary for a species to survive in a continuously changing environment. At

the same time such mutation defines the typical lifespan of the somatic cells and the organism as a whole.

In many respects this monograph provides the biological counterpart to Burch's more mathematical treatment of the same problem (*An Inquiry Concerning Growth, Disease and Ageing*; Oliver and Boyd, Edinburgh, 1968), but Sir MacFarlane has been able to draw on the mass of material which has been published since 1968. Of the references quoted, 60% are drawn from works published during the last six years. And Sir MacFarlane's approach is an improvement on that of Burch, in that it discusses the involvement of environmental factors.

Drawing facts from studies of both microbiological and mammalian cell lines, Sir MacFarlane suggests that errors introduced into DNA by faulty expression of those enzyme systems which control the repair of the nucleic acid chains may determine not only the life span of the organism, but also the incidence of age-mediated lesions. These, because of failures in the normal surveillance mechanisms of the body—age related reductions in T and B cell populations—may induce a degree of error accumulation similar in many respects to that which, according to Orgel, results from faulty protein synthesis.

Once again Sir MacFarlane has presented us with a monograph which is both thought provoking and on which future experimental work may be based. All-in-all this book is well worth reading, and it will be quoted often as the study of the ageing process develops.

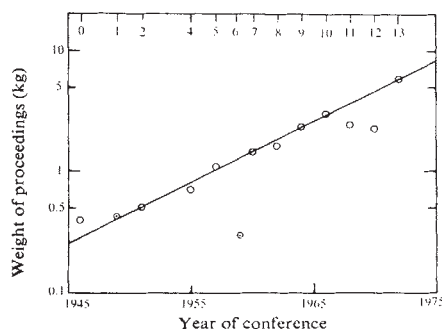
David A. Hall



Aonyx capensis, the cape clawless otter. From *The Carnivores of West Africa*. By D. R. Rosevear. Pp. vii+548+11 plates. (British Museum—Natural History: London, 1974.) £18.50.

Weighing words in conference proceedings

"I predict for the suitably apocalyptic year of 1984 that ... LT 17 will take place, its proceedings will weigh half a hundredweight, and they will take over five years to come out."



THE series of international conferences on low temperature physics has come to be known as 'LT' conferences. The accompanying graph is a log-linear plot of the weight of those LT Proceedings which I have access to, as a function of time and showing the LT number (given along the top abscissa). (On my reckoning, incidentally, the first LT was held at Cambridge in 1946 and not, as the Organising Committee of LT 13* state, in 1949; it is a very fascinating work, well worth reading, and should perhaps be incorporated into the numerology as LT 0). There are several anomalies which can be readily explained. LT 11 was even more anomalous than is obvious from the graph, for a combination of reasons: it came out quickly (within about five months), quite cheaply (about \$17.00 for two volumes) and included some useful discussion. Other people may choose to draw different lines through the points on the graph, depending on their judgement of possible weighting factors. From my own exponential growth line (and some other input data), I predict for the suitably apocalyptic year of 1984 that, unless some serious steps are taken, LT 17 will take place, its proceedings will weigh half a hundredweight, and they will take over five years to come out.

If it had ever been worthwhile—or possible—to review in adequate detail the contents of these four volumes, which it probably never was, it is certainly not worth while now. The LT 13 conference took place in Boulder, Colorado, well over two years ago (August 1972), and the proceedings have only been saved from the ultimate embarrassment of not appearing before the next conference by the fact that LT 14 will take place three years after LT 13 instead of two, as was the previous custom. Colleagues and friends who have been responsible for producing this almost unbelievably ex-

pensive work of supererogation have had a very hard time of it indeed—a somewhat hilarious time too (for those who were not involved, at least)—which included the hijacking of the plane carrying some of the manuscripts, and its destruction in a Middle Eastern desert. They are to be congratulated and commiserated with on a task which, as far as I am concerned, will be thankless.

Is it really worth all the effort and cost to produce in four beautiful volumes all these contributions which, if valuable enough ought to be made more readily available in regular journals, and if not, ought to sink unobtrusively into oblivion? Short conference contributions are not usually, and certainly should not necessarily be, as complete as papers published in the normal way, and one is never certain when referring to them that they are quite reliable. If they are complete and reliable, and are not published elsewhere because the authors consider them to be published already as proceedings, they are often ignored or forgotten.

The four volumes reviewed here contain more than 500 papers on a vast range of topics—all low temperature aspects of quantum fluids and solids, metals, instrumentation, techniques, and so on—totalling over 2,500 pages. The admirable aim of the organisers was to allot half of the available time to plenary sessions dealing with new developments but of interest to a general audience. The plenary lectures were certainly successful but because of the large number of parallel sessions with contributed papers the plenary papers form only about 3% by number and 6% by pages of the proceedings. In addition, there were special sessions, evening sessions, and discussions, much of which were more interesting and valuable than the contributed work, but not recorded. It is very unfortunate, for example, that probably the most interesting topic, which occupied a considerable amount of private and public discussion time (the newly discovered superfluidity in liquid He³) should be found only cursorily reported

under 'Quantum Crystals'. I should like to suggest that the only rational way of dealing with the record of a conference of this size and diversity is to make available at the conference itself fairly detailed abstracts of the papers and properly to publish as a book only a collection of the invited papers whose authors would be aware that they were contributing to a book and not to ephemeral conference proceedings. Everybody would be saved a lot of time, trouble and probably expense, and the result would be much more useful.

Now that the time and effort have, however, been spent it would be unfair not to say that there are many interesting and useful things in the books, as may readily be found by reading through the list of contents. One really astonishing thing is the enormous amount of work on liquid helium, occupying about a quarter of the whole and quite enough for a conference on its own. None of the material can be used, of course, without recent confirmation by ordinary publication or by private communication, with certain exceptions. The exceptions are the established material in the plenary session lectures (each only slightly longer than a letter in *Phys. Rev. Lett.*, although the talks themselves lasted nearly an hour); and, most interestingly from an historical point of view, A. A. Abrikosov's address as recipient of the Fritz London Award (given, alas, *in absentia*) which contains a brief though generous account, which I had come across only by hearsay before, of the way in which Landau, perhaps unintentionally, discouraged him in 1953 from publishing his discovery of vortices in the mixed state of Type II superconductors.

So the conclusion of all this is that all institutions involved with low temperature physics will, if they can afford it, have to buy these books in order to keep their reference literature complete. But it should never happen again, and I sincerely hope that the Organising Committee of LT 14 take note. See the graph. **D. F. Brewer**

The Social Behaviour of the Bees, by Charles Michener, which was reviewed in *Nature*, **253**, 75; 1975, is available in Britain through Harvard University Press, 126 Buckingham Palace Road, London, SW1, at £12.50.

In the same edition (**253**, 76; 1975) we published a review of *Our Future Inheritance: Choice or Chance?* This book was written by Alun Jones and Walter F. Bodmer, whose names were omitted from the bibliographical details.

*Low Temperature Physics-LT 13. Vols 1-4. Edited by K. D. Timmerhaus, W. J. O'Sullivan and E. F. Hammel. Pp. xv+669. (Plenum: London and New York, 1974.) \$42.00 each.

obituary

William D. Coolidge, the developer of the modern X-ray tube and the ductile tungsten filament used in electric light bulbs, has died in Schenectady, New York. He was 101 years old.

Born in Hudson, Massachusetts, Dr Coolidge graduated from the Institute of Technology there in 1896, specialising in physical chemistry and electrical engineering. After obtaining his doctorate in 1899 at the University of Leipzig, he joined the research staff of General Electric. In 1908, he invented a method of working tungsten, one of the most brittle of metals, while it was hot, thus making it possible to draw out the metal into wires much thinner than human hair. The durable thin filaments made by this method led to a more durable light bulb for use in cars and trains, and to more powerful and more portable X-ray equipment. The X-ray tube invented in 1913, the 'Coolidge tube', is still the model on

which modern tubes are based. In this case, a hot tungsten filament replaced a cold aluminium cathode. Among many awards were an honorary MD from the University of Zurich, the Rumford Medal of the American Academy of Arts and Sciences, and the Howard N. Potts Medal of the Franklin Institute. Dr Coolidge will be honoured by the National Inventors Hall of Fame at the Patent and Trademark Office, from which he has received 83 patents. In 1932, he was appointed director of the General Electric Research Laboratory. Under his direction, many new discoveries were made, including the development of the sodium vapour lamp, high-quality magnetic steel, improved ventilating fans and the electric blanket. On the occasion of his 100th birthday, he was presented with a huge birthday cake lit with 100 ductile-tungsten lamps and topped with part of a large X-ray tube.

William Charles Osman-Hill, the primate anatomist, has died at the age of 74.

After graduating from Birmingham University in 1925 with an MD, Dr Osman-Hill became an assistant lecturer in zoology there and later lecturer in Anatomy from 1925-30. He was professor of anatomy in Ceylon University until 1944 and became a reader in physical anthropology in the University of Edinburgh from 1945-50. From 1950-62, he was Professor to the Zoological Society of London and from 1962 until the time of his death he was one of the Trustees of the Hunterian Collection in the Royal College of Surgeons of England, of which he was also a Fellow. Dr Osman-Hill will be remembered particularly for his work on primate anatomy, about which he has published several books, and for many enlightening papers on primate evolution and anthropology.

announcements

Awards

Michel Crozon and **Peter Sonderegger** have been awarded the **Prix Scientifique** by the Fondation de France, for their work on 'Physics for the Man in the Street' at Aix-en-Provence in 1973.

John Miller Meek has been awarded the **Faraday Medal**, for research into electrical discharges in gases, and in particular the mechanism of the electric spark.

E. Kodicek has been awarded the **CIBA Medal and Prize** by the Biochemical Society for contributions to the study of metabolism and mode of action of cholecalciferol in animal tissues.

D. R. Trentham has been awarded the **Colworth Medal** by the Biochemical Society for his work on the kinetics of enzyme action and in particular his contribution to the transient kinetics of myosin-ATPase.

Appointment

Kenneth Dolder has been appointed to the chair of atomic physics at the University of Newcastle upon Tyne.

International meetings

March 13-14, **Chromosomal Proteins and their Role in Regulation of Gene Expression**, Florida (Florida Colloquium on Molecular Biology, Department of Biochemistry, University of Florida, Gainesville, Florida 32610).

April 7-9, **Food from Waste**, Weybridge, Surrey (The Secretary, National College of Food Technology, Weybridge, Surrey, UK).

April 13-16, **Energy and Environment**, California (Betty Peterson, Executive Director, Institute of Environmental Sciences, 940 East Northwest Highway, Mount Prospect, Illinois 60056).

April 14-17, **Magnetics**, London (Intermag 75, Institute of Physics, 47 Belgrave Square, London SW1X 8QX, UK).

April 21-25, **Nuclear Energy**, Paris (P. Zaleski, CNE-Inscriptions, B.P. No. 27 92140 Clamart, France).

May 6-8, **Quantitative Magnetospheric Models**, Southern California (W. P. Olson (Chairman), McDonnell Douglas Astronautics Company, 5301 Bolsa Avenue, Huntington Beach, California 92647).

May 11-14, **Ozone for Water and Wastewater Treatment**, Montreal, Canada (The Symposium Chairman, 24 Central Avenue, Waterbury, Connecticut 06702).

May 11-16 **Calcium Transport in Contraction and Secretion**, Padova, Italy (F. Clementi, Istituto di Farmacologia, Università di Milano, 32 via Vanvitelli, 20129 Milan, Italy).

May 12-14, **Transplantation and Clinical Immunology**, Lyon (Secretariat, Docteur R. Triau, Fondation Mérieux, 17 rue Bourgelat, 69002 Lyon, France).

May 12-16, **Acid Precipitation and the Forest Ecosystem**, Columbus, Ohio (Dr Leon S. Dochinger, US Forest Service Laboratories, P.O. Box 365, Delaware, Ohio 43015).

Reports and publications

Great Britain

The Soal-Goldney Experiments with Basil Shackleton: a Discussion. By Christopher Scott, et al. (Proceedings of the Society for Psychical Research, Vol. 56, Part 209, October 1974.) Pp. 41-174. (London: Society for Psychical Research, 1974.) [1612]

University of Hull. Annual Report, 1973/1974. Pp. v + 115. (Hull: The University, 1974.) [1612]

UKAEA, Harwell. Radioactive Fallout in Air and Rain: Results to the Middle of 1974. By R. S. Cambray, J. D. Eaking, Miss E. M. R. Fisher and D. H. Peirson. Pp. 48. (London: HMSO, 1974.) £1 net. [1612]

Report of the Social Science Research Council, April 1973-March 1974. Pp. 66. (London: HMSO, 1974.) 71p net. [1812]

Social Science Research Council. Research Supported 1974. Pp. 196. (London: Social Science Research Council, 1974.) 90p net. [1812]

The British Library. First Annual Report 1973/1974. Pp. 16. (London: The British Library, Store Street, WC1E 7BY, 1974.) [1912]

Natural Environment Research Council. Report of the Council for the period 1 April 1973-31 March 1974. Pp. v + 153 + 15 photographs. (London: HMSO, 1974.) £1.50 net. [2012]

The Mental Health Trust and Research Fund. Annual Report for 1974. Pp. 28. (London: The Mental Health Trust and Research Fund, 8 Wimpole Street, W1, 1974.) [2012]

Department of Agriculture and Fisheries for Scotland. Farming the Red Deer. (The first report of an investigation by the Rowett Research Institute and the Hill Farming Research Organization.) Pp. 93 + 14 plates. (Edinburgh and London: HMSO, 1974.) £1.70. [2012]

Proceedings of the Royal Irish Academy, Vol. 74, Section A. No. 15: Paired Homotopy Type. By T. Porter. Pp. 103-114. 32p. No. 16: On Separation Axioms Weaker Than T_1 . By D. M. G. McSherry. Pp. 115-118. 18p. No. 17: The Viral Theorem and Schwinger's Variational Principle in Collision Theory. By J. D. G. McWhirter and B. L. Moiseiwitsch. Pp. 119-131. 36p. Vol. 74, Section B. No. 25: Granophyre Net-Veining in the Dolerites of Slieve Gullion. By R. W. D. Elwell, P. M. Bruck and P. J. O'Connor. Pp. 439-453 + plate 14. 32p. No. 26: Anticancer Agents-X. Cyclisation of 1-Acyl-, 4-Alkylthiosemicarbazide Derivatives to 1,2,4-Triazole-3-Thiones in the Presence of Hydrazine. By G. N. O'Callaghan. Pp. 455-461. 16p. No. 27: *Amphitholina cuniculus* (Stebbing), a Little-known Marine Amphipod Crustacean New to Ireland. By A. A. Myers. Pp. 463-469. 15p. (Dublin: Royal Irish Academy, 1974.) [2312]

Wildfowl 25. Edited by G. V. T. Matthews and M. A. Ogilvie. Pp. 176. (Oxford and London: Blackwell Scientific Publications, 1974. Published for the Wildfowl Trust, Slimbridge.) [2312]

Bulletin of the British Museum (Natural History). Entomology. Vol. 31, No. 5: The *Nesothrips* Complex of Spore-Feeding Thysanoptera (Phlaeothripidae: Idolothripinae). By L. A. Mound. Pp. 107-188. (London: British Museum (Natural History), 1974.) £4.80. [2412]

Office of Population Censuses and Surveys. The Current Tempo of Fertility in England and Wales. By Dr. S. M. Farid. (Studies on Medical and Population Subjects, No. 27.) Pp. 95. (London: HMSO, 1974.) £5.30 net. [3112]

Ministry of Agriculture, Fisheries and Food. Agricultural and Food Statistics: a Guide to Official Sources. (Studies in Official Statistics, No. 23.) Pp. vi + 84. (London: HMSO, 1974.) £2 net. [3112]

Methods of Chemical Analysis of Iron and Steel. Pp. xi + 161. (Sheffield: British Steel Corporation, Corporate Development Laboratory, 1974.) £4. [3112]

Other countries

The Walter and Eliza Hall Institute of Medical Research, 1973/1974. Pp. 152. (Parkville, Victoria: The Walter and Eliza Hall Institute of Medical Research, 1974.) [2511]

CERN-European Organization for Nuclear Research. CERN 74-22: Proceedings of the 1974 CERN School of Physics, Windermere, England, 16-29 June 1974. Pp. vii + 215. (Geneva: CERN, 1974.) [2611]

Publications of the World Health Organization, 1968-1972—a Bibliography. Pp. 158. (Geneva: WHO, London: HMSO, 1974.) Sw.fr.20. [2611]

World Health Organization. Public Health Papers, No. 58: Suicide and Attempted Suicide. Edited by Eileen M. Brooke. Pp. 127. (Geneva: WHO; London: HMSO, 1974.) Sw.fr.8. [2711]

North of Latitude Eighty: The Defence Research Board in Ellesmere Island. By G. Hattersley-Smith. Pp. ix + 121. (Ottawa: Information Canada, 1974.) \$6.75. [212]

Geological Survey of Canada. Guide to the Geology of Riding Mountain National Park and Its Vicinity: History of Its Upland and Other Scenery. By A. H. Lang. (Miscellaneous Report No. 20.) Pp. 68. (Ottawa: Information Canada, 1974.) \$2. [212]

Bulletin of the American Museum of Natural History, Vol. 154, Article 1: The Eyeless Beetles of the Genus *Arianops* Brendel (Coleoptera: Pselaphidae). By Thomas C. Barr, Jr. Pp. 1-52. (New York: American Museum of Natural History, 1974.) \$2.25. [212]

Lawrence Berkeley Laboratory: Research Highlights 1974. Pp. 60. (Berkeley, California: Public Information Department, University of California, 1974.) [212]

Smithsonian Contributions to Paleobiology, No. 20: Ultrastructural Studies on Graptolites, 1: The Periderm and Its Derivatives in the Dendroidea and in *Mastigograptus*. By Adam Urbanek and Kenneth M. Towse. Pp. 48. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) \$1.35. [212]

Person to Person

Animals in Research. Professor D. H. Smyth is to carry out an investigation into the contribution made to medical problems by tissue culture, computers and other methods not involving living animals, at the suggestion of the Research Defense Society. He would welcome suggestions and information from individuals or societies (MRC Unit for Metabolic Studies in Psychiatry, Middlewood Hospital, Sheffield, UK).

Youth and Universe. The West Yorkshire branch of the British Association of Young Scientists is organising a four-day programme of lectures and films on the theme 'The International Animal'. This gathering of several hundred school and university/college students from April 1-4 at the University of York will consider such topics as the evolution of man and the future of man in the universe. Contact (before March 1): Bootham School, York YO3 7BY, UK).

Romantic Science. Researcher interested in renaissance of artistic activity during post-Napoleonic or Romantic Period in Germany and Austria (mid-19th to early 20th century) would be grateful for any information about similar developments at that time in science (Robbie Vickers, 49 Barry Road, East Dulwich, London SE22, UK).

Egyptian Medicine. Student of Egyptology at Oriental Institute, Free University, Brussels, specialising in problem of 'doctor-medicine preparer' (his role, place in society, knowledge), and of Egyptian pharaonic medicine in general, wishes to communicate with doctor interested in research in history of medicine (Martine La Graviere, 10 rue Philippe Dewolfs, B1170 Bruxelles, Belgium).

Irish Astronomy. Any information on Irish megalithic sites would be gratefully received (Lillian Thompson, Flat 3, 14 College Terrace, Brighton BN2 2EE, Sussex, UK).

Flat or small house. Required for a week at the end of July in the vicinity of Bruges, Ghent or Rheims. Two adults and baby (R. E. Woodham, 167 Crofton Road, Orpington, Kent BR6 8JB, UK).

There will be no charge for this service. Send items (not more than 60 words) to Robert Vickers at the London office. The section will include exchanges of accommodation, personal announcements, and scientific queries. We reserve the right to decline material submitted.

Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft, Berlin-Dahlem. Heft 160: Untersuchungen über die Anfälligkeit verschiedener Getreidearten gegen den Erreger der Schwarzbeinigkeit, *Ophiobolus graminis* Sacc. Von Dr. Horst Mielke. Pp. 61. (Berlin-Dahlem: Biologische Bundesanstalt für Land- und Forstwirtschaft, 1974.) DM9. [312]

World Health Organization Technical Report Series. No. 556: Detection of Dependence-Producing Drugs in Body Fluids—Report of a WHO Meeting of Investigators. Pp. 50. (Geneva: WHO; London: HMSO, 1974.) Sw.fr. 5. [412]

Annals of the South African Museum. Vol. 65, Part 10: The Cranial Morphology of *Thrinacoselache* Seeley. By S. Fourie. Pp. 337-400. R.8.40. Vol. 65, Part 11: Aspects of the Biology and Ecology of the Genus *Tyllos* Latreille. By Brian Kensley. Pp. 401-471. R.8.70. Vol. 66, Part 1: Population Structure in *Agamix* atra and *Cordylus cordylus* in the Vicinity of the Kelders, C.P. By Bryan R. Burrage. Pp. 1-23. R.4. Vol. 66, Part 2: A New South African Representative of the South West African Genus *Namibinyllus* Hesse (Diptera: Mydidae), with some Ecological Notes on the Habits of the Species. By A. J. Hesse. Pp. 25-34. R.2. Vol. 66, Part 3: The Cranial Morphology of the Lower Triassic Dicynodont *Myosaurus gracilis*. By Michael A. Cluver. Pp. 35-54. R.3.80. (Cape Town: South African Museum, 1974.) [512]

Preliminary Report on the Izu-Hanto-oki Earthquake of 1974. (Special Bulletin of the Earthquake Research Institute, University of Tokyo. No. 14.) Pp. 255. (Tokyo: Earthquake Research Institute, University of Tokyo, 1974.) [612]

Twenty-second Annual Report of the Australian Atomic Energy Commission, 1973/1974. Pp. 117. (Coogee, NSW: Australian Atomic Energy Commission, 1974.) [912]

Rheinisch-Westfälische Akademie der Wissenschaften. Vorträge Nos. 236 und 239. (Düsseldorf: Westdeutscher Verlag, 1974.) [912]

Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Bulletin 229: Metamorphic and Plutonic Rocks of Northernmost Ellesmere Island, Canadian Arctic Archipelago. By Thomas Frisch. Pp. 87. \$4. Paper 74-14: Palynology of an Upper Cretaceous Section, Horton River, District of Mackenzie, N.W.T. By D. J. McIntyre. Pp. 56 (24 plates). \$3. Paper 74-30: Offshore Geology of Eastern Canada. Vol. 1. Edited by B. R. Pelletier. Pp. 160. \$6.50. Paper 74-56: The Relationship Between Mercury Occurrence and Mining Activity in the Nottaway and Rupert River Basins of Northwestern Quebec. By J. E. MacLachy and I. S. Jonasson. Pp. 10. \$2. (Ottawa: Information Canada, 1974.) [912]

World Meteorological Organization. Technical Note No. 134: Review of Urban Climatology, 1968-1973. By Dr. T. R. Oke. Pp. xviii + 132. (Geneva: World Meteorological Organization, 1974.) [1112]

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